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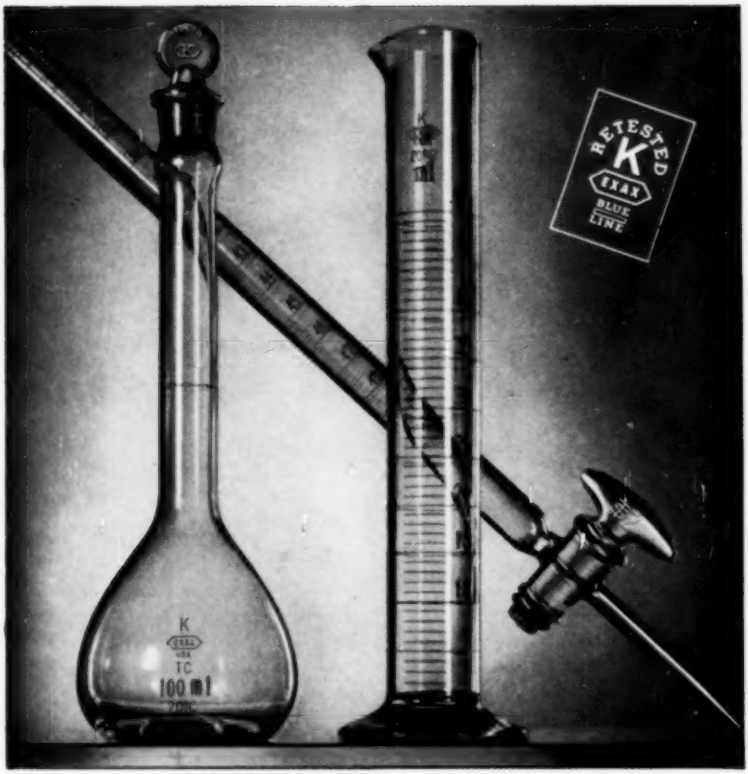

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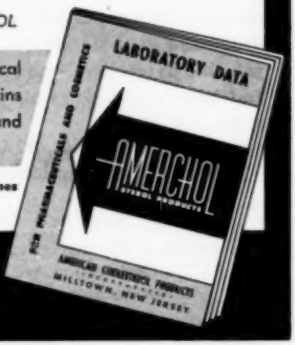

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JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVIII | *NOVEMBER, 1949* | NUMBER 11 | CONSECUTIVE NO. 22

The Colorimetric Estimation of N(2-Pyridyl) Substituted Anti-Histamine Drugs*,†

By HURD M. JONES‡ and EDWARD S. BRADY§

Tripelennamine with cyanogen bromide in a solution of potassium acid phthalate forms, upon the subsequent addition of aniline, a yellow-colored compound which obeys Beer's law. This reaction may be used to determine from one-half to ten mg./ml. of tripelennamine (Pyribenzamine) in solution. Aqueous extracts of the tablets, elixir, and cream gave results consistent with other methods of assay. Urinary excretion of tripelennamine after oral administration was also estimated by this colorimetric method. The extension of this method to the assay of other N(2-Pyridyl) substituted antihistamines in pharmaceuticals and urine has shown promising results, and colored compounds suitable for colorimetric estimation have been obtained with thenylpyramine (Histadyl) and pyranisamine (Neo-Antergan).

KÖNIG observed the formation of a colored compound when pyridine was reacted with cyanogen bromide and aniline (1). This reaction has been employed for the colorimetric quantitative determination of nicotinic acid (2, 3). Others have employed *p*-methylaminophenol (4) and *p*-aminoacetophenone (5) in place of the aniline with satisfactory results. Workers have indicated that this reaction is less valuable in the assay of pyridyl derivatives having substitutions on the alpha position (6). However, since the commonly used methods of analysis of 2-pyridyl substituted antihistamine drugs are somewhat slow, often involving the precipitation of the base as the dipicrate (7), an investigation of the König reaction as applied to this group of compounds was undertaken. Those antihistamine compounds which contain the pyridyl group as a part of their structure are tripelennamine (Pyribenzamine—Ciba), thenylpyramine (Histadyl—Lilly), methapyrilene (Thenylene—Abbott), pyranisamine (Neo-Antergan—Merck), prophenpyridamine (Trimeton—Schering), and doxylamine (Decapryn—Merrell). Tripelennamine hydrochloride was used for most of the preliminary work, but the other antihistamine drugs listed respond in a similar manner to give colored compounds which obey Beer's law and may be quantitatively determined by this method.

* Received April 30, 1949, from the School of Pharmacy, University of Southern California, Los Angeles.
Presented to the Scientific Section, A. PH. A., Jacksonville meeting, April, 1949.
† This paper is based, in part, on a thesis presented to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of M.S. in Pharmacy.
‡ Present Address: Texas State University for Negroes, Houston, Tex.
§ School of Pharmacy, University of Southern California, Los Angeles.

EXPERIMENTAL

Reagents.—A stock solution of tripelennamine hydrochloride 1 mg./ml. was used for the preparation of standard dilutions. Solutions of the tablets, capsules, and elixir were prepared by simple solution, and those of the cream and ointment by extraction with water; the final volume was adjusted so that the concentration approached that of the standard. Reagents employed were: cyanogen bromide, 4% aqueous solution; aniline, 4% in alcohol; and potassium acid phthalate, 2% aqueous solution.

Procedure.—To 3 cc. of the standard dilutions, or of the solution to be assayed, representing concentrations of from one-half to 3 mg. of the drug, 1 cc. of cyanogen bromide solution and 5 cc. of potassium acid phthalate solution were added, and, after fifteen minutes, 1 cc. of the aniline solution. After twenty more minutes, readings were taken on the Klett-Sommerson photoelectric colorimeter, using a blue (400–465 mμ) filter. The zero setting was determined in the usual manner, employing 3 cc. of distilled water in place of the tripelennamine solutions, and adding the reagents as listed above. Results obtained from standard solutions and various preparations are shown in Fig. 1 and Table I.

TABLE I.—COMPARISON OF ANALYTICAL METHODS

Material	Theoretical Content	Precipitation Method	Colorimetric Method
Tripelennamine	2.0	2.0	2.0
PBZ tablets	2.0	2.0	2.0
PBZ elixir	2.0	1.94	2.1
PBZ cream	2.5	2.54	2.54

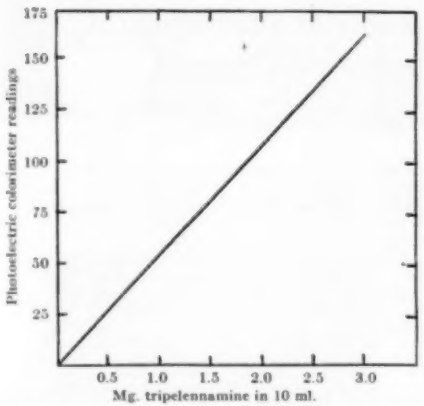

Fig. 1.—Calibration curve for tripelennamine.

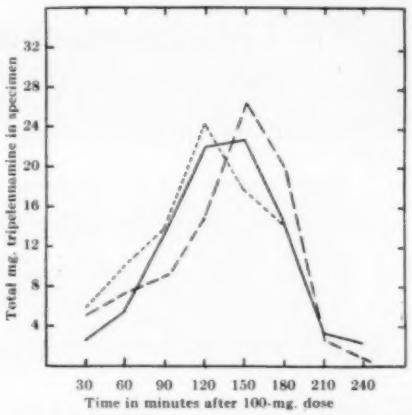

Fig. 2.—Urinary excretion of tripelennamine.

Comment.—Although the optimum *p*H for this reaction in the determination of nicotinic acid has been established at 5.5 to 7.5 (6), precipitation occurred in the antihistamine solutions when a phosphate buffer of *p*H 7 was used. A stable system resulted at *p*H of 6, but the color, which developed more rapidly, also faded rapidly after the maximum intensity was reached. For that reason, the potassium acid phthalate solution used proved the most satisfactory, although a further investigation of the influence of buffer solutions and *p*H on this procedure is contemplated.

Utilization of this method of analysis to investigate the rate of urinary excretion of tripelennamine was briefly undertaken. In four cases, 86% to 91% of a 100-mg. dose was apparently excreted in four and one-half hours; in three of these cases, with specimens collected at half-hour intervals, maximum excretion occurred two to three hours after administration (Fig. 2). Although it is apparent that other pyridine compounds which occur in the urine (including nicotinic acid) would probably give results over longer periods indicating a greater than 100% recovery of the antihistamine administered, this method may still prove of value in the study of the rate of excretion of these compounds.

SUMMARY

A colorimetric method for the determination of N(2-pyridyl) substituted antihistamine drugs has been presented. The opening of the pyridine ring by cyanogen bromide and the coupling of the intermediate compound with aniline results in the formation of a colored complex which follows Beer's law. This method may be applied to the analysis of pharmaceutical products and urinary excretion of six proprietaries of this class.

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The Evaluation of the Glycoside, Oleandrin, on the Embryo Chick Heart*

By MORRIS M. WOLFRED

Embryonic chick hearts of 48- to 50-hour development were dissected by known methods and exposed to glycosidal-Tyrode solutions in prewarmed chambers of 39°. Atrioventricular block took place in the majority of instances and was taken as the end point. Occasionally dropped beats or diastolic arrest occurred first, and in such cases these were taken as the end point. Twelve hearts were used for five concentrations of the drug. The length of the complete systole-diastole heart cycle, measured in seconds, was taken at minute intervals. The pharmacologic evidence indicated that the glycoside, oleandrin, has a cardiotonic action on the embryo chick heart. The results follow a quantitative pattern in relation to the speed of the complete heart cycle to the potency of the drug, the end result demonstrating cardiac fibrillations ending in atrioventricular block.

A METHOD is adopted for a rapid means of evaluating the therapeutic activity of the glycoside, oleandrin, obtained from *Nerium oleander*, on the embryonic chick heart. This procedure may also be applied as a means of bioassay.

HISTORY

Nerium oleander Linné, a native plant of Asia, has been introduced into the United States and many of the warmer countries of the world. In the northern part of the United States, it is sometimes grown as a flowering house plant. Oleander, which belongs to the Apocynaceae family, was described in the writings of Hippocrates and other ancient documents, indicating that knowledge of its poisonous properties is very old. The plant is highly toxic in the green as well as the dry state. Cattle, horses, sheep, and chickens have been poisoned by consuming the leaves. A few cases of poisoning have been reported among human beings, including a contact dermatitis. The symptoms of poisoning were described as nausea, vomiting, colic, vertigo, drowsiness, decreased pulse rate, irregular heart action, marked mydriases, bloody diarrhea, unconsciousness, respiratory paralysis, and finally, death (1).

The poisonous glycosides of oleander display properties similar to those of the digitalis and strophanthus glycosides. The chemical structure of these cardiac glycosides shows that they are mostly hydroxylactones of the sterol hydrocarbons in which one hydroxyl group is attached to a carbohydrate molecule or to a chain of several carbohydrate molecules.

On early investigation the active principle of *Nerium oleander* was thought to be an alkaloid. However, further research demonstrated that a glycoside called oleandrin, obtained from the leaves, was the main constituent. Oleandrin rotates the plane of polarized light to the left and is represented by the molecular formula $C_{31}H_{48}O_9$. This compound is resistant to dilute acids and is slowly split into an aglycone called oleandrigenin, to which previously the formula $C_{23}H_{34}O_6$ was given, but was shown to have the composition $C_{23}H_{32}O_6$. Oleandrigenin was obtained by treating gitoxigenin with acetic anhydride in pyridine at 0°. When heated with hydrochloric acid in an aqueous solution of methyl alcohol, oleandrin yielded the aglycone oleandrigenin plus the sugar oleandrose. The constitution of oleandrigenin and oleandrin was established in the following manner: oleandrigenin was oxidized with chromic acid in glacial acetic acid to oleandrigenone with a melting point of 250° to 252°, which gave, on treatment with concentrated hydrochloric acid, dianhydrooleandrigenone. The latter was proved identical with digitalgenone by the mixed melting point and the absorption curve (2). These results show that oleandrigenin contains at the third carbon a free hydroxyl group which was oxidized to a ketone group and the acetyl rest was at the sixteenth carbon. Oleandrin contains, therefore, the sugar oleandrose, attached to the third carbon. As proved, oleandrin is very closely related to the digitalis groups.

Mention is made in the literature of an alkaloid found in oleander by Schmiedeberg (3), which he named neriine. This substance exists in the form of a yellowish white powder, which is very bitter, nonhygroscopic, extremely soluble in water, alcohol, and acetone, and insoluble in ether, chloroform, and benzine.

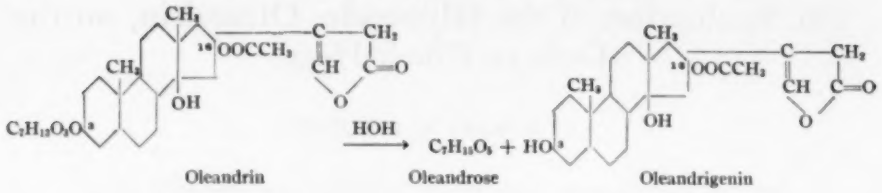
Chen, Anderson, and Robbins (4), reported the action of acetyl-free oleandrin which occurs in nature and is known as desacetyl-oleandrin. They found desacetyl-oleandrin to be more prompt and less persistent in activity than oleandrin on cats and frogs.

The glycoside oleandrin, $C_{31}H_{48}O_9$, has the following physical properties: white needles from 50% alcohol, practically insoluble in water and methyl alcohol, soluble in alcohol. ether, and chloroform, a melting point of 249° to 250°, and $[\alpha]_D$ −45.95 (5).

EXPERIMENTAL

Extraction of Oleandrin.—Distribution of cardiac glycosides in various plants varies quantitatively with the portion of the plant used as raw material for their isolation. The leaves of *Nerium oleander* were picked between September and January from mature trees growing on the Pacific coast and con-

* Received May 23, 1949, from the Department of Pharmacology, School of Pharmacy, University of Southern California, Los Angeles.



tained no signs of degeneration or fungus growth. The leaves were chosen for this work because experimental evidence proved that they contained the highest percentage of glycoside. The leaves were coarsely ground and their expressed juice added to the ground drug. They were then placed in a percolator, macerated for twenty-four hours, and the glycoside extracted by means of an aqueous alcoholic extract as a menstruum. The tannins were precipitated with lead subacetate and separated by filtration. The excess lead was eliminated from the filtrate by means of sodium oxalate. A neutral salt such as ammonium sulfate was used to precipitate out the glycoside, which was then filtered and separated by dissolving in a 50% aqueous alcoholic solution. The glycoside was then extracted from the alcoholic solution by the use of chloroform. The chloroform was evaporated and the glycoside crystallized from an alcoholic solution. The average yield of oleandrin was 0.08%. Experimental progress in the extraction was traced by means of a microscope, which was used in identifying the purified crystals. Fractional crystallizaton was also undertaken in obtaining the glycosides in pure state. Melting points were used to confirm the crystals.

Action on Embryo Chick Heart.—Embryonic chick hearts of 48- to 50-hour development were dissected out by the following procedure: A window was cut in the shell of the egg by first drilling a small hole and widening the orifice with a pair of scissors until the yolk was exposed. The albumen was withdrawn by means of suction and the embryo dissected by the method of Paff (6, 7), in which the embryo was lifted from the yolk, transferred to a Tyrode solution, and placed under a dissecting microscope. The amnion was removed first, the conus and the sino-atrium dissected and placed in a well of Tyrode solution, and a slip cover placed over the well. The solution was prewarmed to 39° prior to immersion of the heart.

The Tyrode solution was made up to give a *p*H of 7.4 according to the following formula (8).

NaCl	18.00
KCl	00.02
$CaCl_2$	00.02
$MgCl_2 \cdot 6H_2O$	00.01
NaH_2PO_4	00.005
$NaHCO_3$	00.028

Stock solutions of Tyrode and oleandrin dissolved in 95% alcohol were prepared. Control experiments showed that Tyrode solutions containing 2% alcohol had no discernible effect on the hearts (9).

At the time of testing, the dissected chick heart was placed in a well-slide filled with Tyrode-oleandrin solution warmed to 39°, prepared as above. The well was covered and the time from immersion of the heart into the solution until the first appearance of cardiac irregularities was measured with a stop watch. Atrioventricular block was, in the great majority of instances, the first reaction, giving evidence of the action of the drug on the heart. Occasionally dropped beats or diastolic arrest occurred first, and in such cases these were taken as the end point. With continuous observation the end point is quite sharp. A series of concentrations were chosen where cardiac irregularities appeared on the average in three to ten minutes. Twelve hearts were used for each of the six concentrations of the drug.

Stop-watch recordings on cardiac irregularities were taken at one-minute intervals, or less in the case of stoppage of the heart. Dilutions of the glycoside were made up accordingly—1:5, 1:10, 1:100, 1:1000, and 1:2000 concentrations. The results are recorded in Tables I to V, inclusive.

TABLE I.—1:5 CONCENTRATION

No.	*A-V* Heart Block in Minutes	Length of Systole-Diastole Cycle in Seconds Taken at Minute Intervals					
		1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	6 Min.
1	3.2	3.3	4.5	4.75	...	...	...
2	3.5	2.6	3.2	3.2	...	...	...
3	3.1	3.0	3.2	3.3	...	...	...
4	3.0	3.1	3.4	...	...	...	...
5	3.3	3.2	3.6	4.6	...	...	...
6	3.4	3.2	3.1	3.5	...	...	...
7	3.3	2.8	3.5	3.7	...	...	...
8	3.3	3.0	4.2	4.0	...	...	...
9	3.0	2.9	...	3.6	...	...	...
10	2.9	3.5	3.6	...	...	...	...
11	3.1	3.3	3.3	3.4	...	...	...
12	3.2	2.9	3.2	3.7	...	...	...
Av.	3.2	3.1	3.5	3.8	...	...	...

TABLE II.—1:10 CONCENTRATION

No.	*A-V* Heart Block in Minutes	Length of Systole-Diastole Cycle in Seconds Taken at Minute Intervals					
		1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	6 Min.
1	3.3	2.8	3.0	3.1	...	...	...
2	4.5	3.1	3.4	3.7	4.1	...	...
3	3.2	2.6	2.9	3.0	...	...	...
4	3.3	3.1	2.9	3.4	...	...	...
5	3.7	..	3.1	3.9	...	...	...
6	3.5	2.9	3.1	3 3	...	...	...
7	3.7	3.1	3.4	3.2	...	...	...
8	3.9	3.1	3.3	3.3	...	...	...
9	3.4	3.3	3.5	3.6	...	...	...
10	3.9	3.0	3.3	3.8	...	...	...
11	4.0	3.2	3.6	3.7	3.9	...	...
12	3.6	3.4	3.5	3.5	...	...	...
Av.	3.7	3.1	3.3	3.6	4.0	...	...

TABLE III.—1:100 CONCENTRATION

No.	*A-V* Heart Block in Minutes	Length of Systole-Diastole Cycle in Seconds Taken at Minute Intervals					
		1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	6 Min.
1	3.6	3.2	3.2	3.3	...	...	...
2	3.7	3.0	3.1	3.1	...	...	...
3	4.0	3.4	3.4	3.4	3.6	...	...
4	4.1	2.9	3.4	3.5	3.7	...	...
5	3.9	3.1	3.3	3.1	...	...	...
6	3.9	3.3	3.6	3.7	...	...	...
7	3.9	2.9	3.0	3.2	...	...	...
8	3.7	3.0	2.9	3.0	...	...	...
9	3.6	3.1	3.3	...	...	...	...
10	3.5	3.3	..	...	...	...	...
11	4.1	3.3	3.5	3.9	3.8	...	...
12	3.8	3.5	3.5	3.7	...	...	...
Av.	3.8	3.2	3.3	3.4	3.7	...	...

TABLE IV.—1:1000 CONCENTRATION

No.	*A-V* Heart Block in Minutes	Length of Systole-Diastole Cycle in Seconds Taken at Minute Intervals					
		1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	6 Min.
1	4.4	3.1	3.0	3.1	3.4	...	...
2	3.2	3.1	2.9	2.9	...	...	...
3	3.6	3.3	3.4	3.7	...	...	...
4	5.2	3.0	3.1	3.7	3.9	3.9	...
5	4.6	3.2	3.2	3.3	3.5	...	...
6	4.1	3.2	3.5	3.6	3.9	...	...
7	3.8	3.2	3.4	3.5	...	...	...
8	4.0	..	3.3	3.4	3.7	...	...
9	3.5	3.4	3.4	3.5	...	...	...
10	4.1	2.9	3.3	3.4	3.7	...	...
11	3.9	3.1	3.4	3.5	...	...	...
12	3.8	3.3	3.2	3.4	...	...	...
Av.	4.0	3.2	3.3	3.4	3.7	3.9	...

TABLE V.—1:2000 CONCENTRATION

No.	*A-V* Heart Block in Minutes	Length of Systole-Diastole Cycle in Seconds Taken at Minute Intervals					
		1 Min.	2 Min.	3 Min.	4 Min.	5 Min.	6 Min.
1	5.0	3.0	3.1	3.1	3.3	3.4	...
2	6.3	2.9	3.0	2.9	3.1	3.3	3.4
3	6.5	3.5	3.6	3.8	3.6	3.6	3.8
4	6.6	3.3	2.9	...	...	3.4	3.6
5	6.3	2.9	2.9	3.0	3.1	3.5	3.7
6	6.1	3.0	3.2	...	3.3	2.9	3.1
7	5.3	2.8	2.9	2.8	3.1	3.3	...
8	5.9	3.2	..	3.1	3.4	3.7	...
9	6.2	3.2	3.2	3.2	3.2	3.2	3.4
10	4.9	3.1	3.3	3.4	3.3	...	...
11	5.3	3.1	3.4	3.7	3.8	4.1	...
12	4.3	3.4	2.8	3.5	3.6	...	...
Av.	5.7	3.1	3.1	3.2	3.3	3.4	3.5

SUMMARY

The glycoside, oleandrin, is closely related to the glycosides of digitalis and hydrolyzes practically identical to the glycoside gitoxin. This similarity in structure accounts for its strong pharmacodynamic action on the embryo chick heart.

The method used for extraction of the glycoside consisted of a combination of experimental and established methods and demonstrated that the glycoside, oleandrin, may be readily obtained in crude form and its purification followed by microscopic method of alcoholic crystallization. The yield of the glycoside, oleandrin, was 0.08 per cent.

The pharmacologic results indicate that oleandrin has a cardiotonic action on the embryonic chick heart, which follows a quantitative pattern in relation to the speed of a complete heart cycle to the potency of the drug, the end result demonstrating cardiac fibrillations ending in atrioventricular block.

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The Antioxidant Effect of 2,2′,3,3′-Tetrahydroxy-5,5′-Dimethyl Biphenyl*

By YUCHUNG CHANG† and OLE GISVOLD‡

The antioxidant effect of 2,2′,3,3′-tetrahydroxy dimethyl biphenyl (T. D. B. P.) was determined in lard, corn oil, and soybean oil, with and without the synergist citric acid, using the Swift stability method. Nordihydroguaiaretic acid (N. D. G. A.) was also tested under the same conditions. T. D. B. P. was shown to be considerably more effective in lard and corn oil than N. D. G. A. In the case of soybean oil, T. D. B. P. was slightly better than N. D. G. A.

IT IS WELL KNOWN that compounds with two or more phenolic hydroxyl groups which are in *ortho* or *para* positions to each other are effective antioxidants. Many of these compounds such as hydroquinone and catechol are too toxic to be used in edible substances for their antioxidant effect. The naturally occurring substance, nordihydroguaiaretic acid, is a very effective antioxidant for animal fats (1) whereas its effectiveness for vegetable oils, like most phenolic antioxidants, is not very great. Even though nordihydroguaiaretic acid contains two catechol groups, its low solubility in water no doubt contributes much to its nontoxicity. The synthesis of several α,ω-bis-(3,4-dihydroxyphenyl)alkanes (2) showed no advantage over nordihydroguaiaretic acid.

No alkylated biphenyls that contain catechol groups in each benzene ring had been previously reported in the literature. Because these compounds should be somewhat readily prepared, a series was synthesized (3) in which the alkyl chain had 1, 3, and 5 carbon atoms, respectively. A preliminary investigation of their antioxidant activities in lard indicated that 2,2′,3,3′-tetrahydroxy-5,5′-dimethyl biphenyl was half again as effective as 2,2′,3,3′-tetrahydroxy-5,5′-dipropyl biphenyl, which, in turn, was as effective as nordihydroguaiaretic acid. This preliminary data warranted a more complete study of the antioxidant activity of 2,2′,3,3′-tetrahydroxy-5,5′-dimethyl biphenyl.

EXPERIMENTAL

The antioxidant properties of 2,2′,3,3′-tetrahydroxy-5,5′-dimethyl biphenyl in fats in varying concentrations with or without the synergist citric acid were tested. Parallel tests with nordihydroguaiare-

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tic acid were made for the purpose of comparison. The tests were carried out by the Swift stability method (4) at 97.7°. In this experiment, however, an oil bath was used instead of the steam bath as was used in the Swift stability method. The oil bath was heated electrically by a resistance wire immersed in the oil, and was conveniently maintained at a constant temperature by a rheostat.

The fatty materials chosen for this work were lard[1] (kettle rendered), corn oil,[2] and soybean oil[3] (edible). The antioxidants were added to the fats directly. The synergist, citric acid, was first dissolved in alcohol, then added to the fats. The alcohol was readily removed by the conditions of the experiment.

The approximate end of the induction period was approached by the method of Stebnitz and Sommer (5). For lard, 1 cc. of 0.01 *N* sodium hydroxide solution was added to the test tube containing methyl red as an indicator and for corn oil and soybean oil, 1.8 and 2 cc. of the sodium hydroxide solution were added, respectively. The development of an orange color in the sample of oil immediately before rancidity was also used to assist in the detection of the end of the induction period (6). This orange color had its origin from the pigments naturally present in these vegetable oils.

The peroxide values of the fats were determined iodometrically by the procedure in the Swift stability method. For lard, the keeping time was expressed as the number of hours of aeration necessary to raise the peroxide value of the sample to 20 milliequivalents of sodium thiosulfate per 1000 Gm. of fat. For corn or soybean oil, the keeping time was the number of hours required to raise this value to 100 milliequivalents. The results were interpreted in terms of the antioxidant index, that is the ratio of the keeping time of fat with antioxidant (with or without the synergist) to that of the unprotected fat. These results are expressed in the following tables.

TABLE I.—PROTECTIVE INDICES IN LARD

Antioxidant Added	Protective Index
0.001% T. D. B. P.[a]	10/3.5 = 2.8
0.001% T. D. B. P. +0.005% citric acid	19.5/3.5 = 5.5
0.002% T. D. B. P.	20/3.5 = 5.7
0.002% T. D. B. P. +0.01% citric acid	33/3.5 = 9.4
0.005% T. D. B. P.	31/3.5 = 8.8
0.005% T. D. B. P. +0.025% citric acid	55/3.5 = 15.2
0.001% N. D. G. A.[b]	8.5/3.5 = 2.4
0.001% N. D. G. A. +0.005% citric acid	18.5/3.5 = 5.2
0.002% N. D. G. A.	16/3.5 = 4.9
0.002% N. D. G. A. +0.01% citric acid	31/3.5 = 8.8
0.005% N. D. G. A.	26/3.5 = 7.4
0.005% N. D. G. A. +0.025% citric acid	45.5/3.5 = 13.0

[a] T. D. B. P. = 2,2′,3,3′-Tetrahydroxy-5,5′-dimethyl biphenyl.
[b] N. D. G. A. = Nordihydroguaiaretic acid.

[1] Kindly supplied by Armour and Company, St. Paul, Minn.
[2] Kindly supplied by the Corn Products Refining Co., Argo, Ill.
[3] Kindly supplied by the A. E. Staley Mfg. Co., Decatur, Ill.

TABLE II.—PROTECTIVE INDICES IN CORN OIL

Antioxidant Added	Protective Index
0.005% T. D. B. P.[a]	18/13 = 1.3
0.005% T. D. B. P. +0.005% citric acid	31/13 = 2.3
0.01% T. D. B. P.	20/13 = 1.5
0.01% T. D. B. P. +0.005% citric acid	35/13 = 2.6
0.02% T. D. B. P.	21.5/13 = 1.6
0.02% T. D. B. P. +0.01% citric acid	40/13 = 3.0
0.005% citric acid	19/13 = 1.4
0.005% N. D. G. A.[b]	13/13 = 1.0
0.005% N. D. G. A. +0.005% citric acid	23.5/13 = 1.8
0.01% N. D. G. A.	13/13 = 1.0
0.01% N. D. G. A. +0.005% citric acid	26/13 = 2.0
0.02% N. D. G. A.	15.5/13 = 1.1
0.02% N. D. G. A. +0.01% citric acid	32.5/13 = 2.5

[a] T. D. B. P. = 2,2′,3,3′-Tetrahydroxy-5,5′-dimethyl biphenyl.
[b] N. D. G. A. = Nordihydroguaiaretic acid.

TABLE III.—PROTECTIVE INDICES IN SOYBEAN OIL

Antioxidant Added	Protective Index
0.005% T. D. B. P.[a]	14.5/11 = 1.3
0.005% T. D. B. P. +0.005% citric acid	18/11 = 1.6
0.01% T. D. B. P.	16/11 = 1.4
0.01% T. D. B. P. +0.005% citric acid	21/11 = 1.9
0.02% T. D. B. P.	19.5/11 = 1.7
0.02% T. D. B. P. +0.01% citric acid	23/11 = 2.0
0.005% citric acid	13.5/11 = 1.2
0.005% N. D. G. A.[b]	12.5/11 = 1.1
0.005% N. D. G. A. +0.005% citric acid	16.5/11 = 1.5
0.01% N. D. G. A.	14.5/11 = 1.3
0.01% N. D. G. A. +0.005% citric acid	19/11 = 1.7
0.02% N. D. G. A.	15.5/11 = 1.4
0.02% N. D. G. A. +0.01% citric acid	21.5/11 = 1.9

[a] T. D. B. P. = 2,2′,3,3′-Tetrahydroxy-5,5′-dimethyl biphenyl.
[b] N. D. G. A. = Nordihydroguaiaretic acid.

SUMMARY

The antioxidant properties of 2,2′,3,3′-tetrahydroxy-5,5′-dimethyl biphenyl in lard, corn oil, and soybean oil showed it to be more effective than those of nordihydroguaiaretic acid. Very promising results were obtained with both lard and corn oil.

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A Comparative Study of the Buffering Capacity of Various Commercially Available Gastric Antacids*

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In order to obtain unbiased information that will serve to rate antacids according to their relative efficiencies, the study reported here was undertaken. Twenty-four of the common gastric antacids of the nonsystemic type were purchased and compared potentiometrically for their relative buffering capacities. The data collected are presented.

THIS STUDY was undertaken in order to make available comparative data on the buffering capacity of a wide selection of commercially available gastric antacids of the nonsystemic type. Although a number of investigators have reported on the efficiency of certain antacids, there is no authoritative comparison of a large list of the more widely prescribed preparations. Such data should be available to the physician and the pharmacist.

Systemic antacids were not included because modern therapy, particularly for prolonged treatment of hyperchlorhydria, seems to favor preparations that are not absorbed into the blood stream.

Choice of method at first was considered to be no problem. The U. S. P. has a procedure for determining the acid-consuming capacity of colloidal aluminum hydroxide and for magnesium trisilicate which are basic constituents of many nonsystemic antacids. The methods are simple and rapid. Several workers have recently pointed out, however, that these tests do not give an accurate picture of antacid function *in situ*. They have proposed more suitable procedures.

Two of the recent modifications are those of Johnson and Duncan (1), and Holbert, Noble, and Grote (2, 3). The following procedure alters somewhat the methods they suggest, but may represent a more informative, yet equally accurate technique.

PROCEDURE

Liquid Antacids.—Twenty-five milliliters of 0.1 *N* HCl is added to 8 ml. of antacid in a 250-ml. beaker and the mixture stirred continuously. The *p*H is recorded immediately, then every five minutes thereafter for the first thirty minutes. To simulate the continuous gastric secretion of HCl, an additional 25 ml. of 0.1 *N* HCl is added to the mixture every thirty minutes. The electrodes of the Beckman *p*H meter (Model H) remain in the mixtures. Readings are taken just before the addition of the HCl. During a test period of 3 hours, 10 readings are taken.

Powdered Antacids or Powdered Tablets.—Twenty-five milliliters of 0.1 *N* HCl is added to the maximum recommended dose, or to any other selected quantity of antacid. The procedure continues from here as for liquid antacids.

EXPERIMENTAL

Twenty-four antacids were compared as to their acid-consuming power by the methods outlined above. Those studied are designated in the following list by composition rather than by trade names. The figures in parentheses, i.e. (1:1), (2:4:1), etc., are the approximate quantitative ratios existing between the active ingredients contained in each.

1. Aluminum Hydroxide Gel
2. Aluminum Hydroxide Tablets
3. Aluminum Hydroxide Suspension
4. Aluminum Hydroxide Tablets
5. Aluminum Hydroxide + Magnesium Trisilicate Suspension
6. Aluminum Hydroxide Gel
7. Aluminum Hydroxide Tablets
8. Aluminum Hydroxide + Magnesium Trisilicate Tablets (1:1)
9. Aluminum Hydroxide + Magnesium Trisilicate Suspension (1:2)
10. Aluminum Hydroxide + Magnesium Trisilicate Tablets (1:2)
11. Aluminum Hydroxide + Magnesium Trisilicate Powder (1:2)
12. Magnesium Trisilicate + Powdered Milk Tablets (1:6)
13. Aluminum Hydroxide + Magnesium Trisilicate Suspension (1:2)
14. Aluminum Hydroxide + Magnesium Trisilicate Tablets (2:1)
15. Aluminum Hydroxide + Magnesium Trisilicate + Mucin Tablets (2:4:1)
16. Aluminum Hydroxide + Magnesium Trisilicate Suspension (1:2)
17. Aluminum Hydroxide + Magnesium Trisilicate Tablets (1:2)
18. Magnesium Trisilicate Powder
19. Magnesium Trisilicate Tablets
20. Magnesium Trisilicate Powder
21. Aluminum Hydroxide + Magnesium Trisilicate Capsules (1:3)
22. Magnesium Trisilicate Tablets
23. Dihydroxy Aluminum Aminoacetate Tablets
24. Inert Plastic Resin Capsules

The comparative data collected are shown in Tables I to V. Attention is particularly called to Table IV showing the additional buffering capacity of fresh homogenized pasteurized milk.

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TABLE I.—*p*H OF MIXTURES OF 0.1 *N* HCl + 8.0 ML. OR 1.0 GM. ANTACID

Time, Min.	Liquids — Max. Recommended Dose (8.0 Ml.) — Sample Number							Powders — 1.0 Gm. — Sample Number			
	1	3	5	6	9	13	16	11	18	20	21
½	2.50	1.81	3.10	2.60	2.80	4.00	4.80	1.20	1.30	1.40	2.20
5	4.20	3.62	4.38	4.10	4.13	4.60	5.79	1.49	1.67	4.34	5.75
10	4.30	4.05	4.45	4.15	4.60	4.78	6.03	1.83	2.09	5.50	6.18
15	4.32	4.21	4.52	4.17	4.88	4.94	6.15	2.20	2.61	6.10	6.34
20	4.36	4.25	4.54	4.20	5.10	5.03	6.23	3.55	3.73	6.28	6.47
30	4.45	4.28	4.59	4.21	5.42	5.14	6.31	6.06	5.35	6.45	6.58
60	4.12	4.15	4.13	3.40	4.23	4.20	4.50	1.77	1.75	6.08	4.90
90	4.05	3.74	3.54	2.45	3.54	4.06	3.96	1.45	1.53	2.80	3.64
120	3.97	2.50	2.85	2.23	2.97	3.90	3.74	1.25	1.44	1.96	3.28
150	3.87	1.89	2.27	2.07	2.26	3.68	3.55	1.15	1.40	1.69	2.75
180	2.85	1.64	1.98	1.83	1.76	3.42	3.29	1.10	1.37	1.55	2.11

TABLE II.—*p*H OF MIXTURES OF 0.1 *N* HCl + 1 GM. ACTIVE INGREDIENTS OF ANTACID TABLETS

Time, Min.	Powdered Tablets — Amount Used (Gm.) — Sample Number											
	1.11	1.54	1.00	1.89	1.80	7.58	1.44	1.41	1.44	1.15	2 Tab.	2 Tab.	4 Cap.
	2	4	7	8	10	12	14	15	17	19	22	23	24
½	1.34	1.30	1.10	1.27	1.20	1.40	1.45	1.18	1.38	1.17	1.20	1.80	1.30
5	2.10	1.80	1.70	2.50	1.93	4.00	3.50	1.90	3.39	1.59	1.60	3.90	5.60
10	2.80	2.31	3.60	3.52	3.48	5.10	3.90	3.10	3.80	2.21	1.90	4.15	6.35
15	3.65	2.98	3.94	3.80	3.70	5.37	4.06	3.59	3.98	4.50	2.23	4.20	6.57
20	3.90	3.95	4.01	3.94	3.87	5.53	4.11	4.52	4.10	5.83	2.90	4.23	6.72
30	4.15	4.10	4.03	4.10	4.22	5.80	4.16	5.80	4.15	6.30	5.15	4.28	6.87
60	3.79	4.05	3.41	3.51	3.54	5.24	3.96	3.65	3.80	5.63	1.90	4.17	4.66
90	3.40	4.00	1.90	3.01	3.14	4.90	3.83	2.45	3.50	2.38	1.72	4.10	2.38
120	2.50	3.96	1.63	2.30	2.56	4.38	3.76	1.80	3.32	1.79	1.62	4.00	1.78
150	2.00	3.95	1.50	2.00	2.03	3.76	3.70	1.67	2.97	1.59	1.53	3.88	1.53
180	1.85	3.92	1.41	1.86	1.23	2.92	2.49	1.58	2.55	1.48	1.48	3.68	1.43

TABLE III.—*p*H OF MIXTURES OF 0.1 *N* HCl + MAXIMUM RECOMMENDED DOSE ANTACID

Time, Min.	Powdered Tablets — Amount Used — Sample Number										
	1 Tab.	1 Tab.	4 Tab.	3 Tab.	4 Tab.	3 Tab.	2 Tab.	2 Tab.	4 Tab.	2 Tab.	3 Cap.
	2	4	7	8	10	12	14	15	17	19	21
½	1.21	1.30	1.40	1.35	1.45	1.40	1.45	1.40	1.65	1.20	2.00
5	1.69	1.48	3.90	3.20	3.94	4.00	4.05	2.60	4.58	2.19	5.95
10	2.15	1.75	4.15	3.80	5.00	5.10	4.24	3.93	5.49	5.50	6.30
15	2.70	2.12	4.20	4.08	5.75	5.37	4.36	4.81	5.90	6.00	6.50
20	3.20	3.14	4.26	4.22	6.11	5.53	4.48	5.45	6.10	6.20	6.60
30	3.95	4.02	4.28	4.48	6.41	5.80	4.82	6.00	6.36	6.51	6.70
60	2.94	4.00	4.14	3.82	5.23	5.24	4.14	5.62	4.48	6.20	5.68
90	2.35	3.95	4.03	3.40	4.18	4.90	4.01	4.00	3.97	5.95	3.75
120	2.02	3.94	3.98	3.20	3.81	4.38	3.97	2.95	3.86	2.80	3.40
150	1.85	3.91	3.90	2.95	3.67	3.76	3.92	2.18	3.82	1.95	3.00
180	1.82	3.90	3.75	2.65	3.58	2.92	3.88	1.83	3.78	1.80	2.55

TABLE IV.—*p*H OF MIXTURES OF 0.1 *N* HCl WITH ANTACID + 60 ML. MILK OR WATER

Time, Min.	Additional Buffering Action of Milk — *A*[a] — *B*[a] — *C*[a] — *D*[a]							
	Milk	H_2O	Milk	H_2O	Milk	H_2O	Milk	H_2O
½	5.25	2.00	5.70	4.20	5.10	1.32	5.00	1.30
5	5.50	2.67	6.10	4.47	5.32	1.98	5.13	2.00
10	5.61	3.28	6.20	4.54	5.37	2.17	5.22	2.55
15	5.69	3.98	6.29	4.62	5.43	2.37	5.29	3.50
20	5.72	4.22	6.35	4.65	5.49	2.63	5.37	4.30
30	5.75	4.35	6.43	4.76	5.68	3.60	5.59	5.60
60	4.44	4.22	5.40	4.23	4.44	1.95	5.13	4.53
90	3.68	3.78	4.68	4.10	3.40	1.73	4.49	3.38
120	3.04	2.40	4.00	3.91	2.54	1.58	3.75	2.58
150	2.52	1.90	3.60	3.66	2.11	1.50	2.88	2.11
180	2.31	1.70	3.30	3.30	1.90	1.30	2.50	1.86

[a] Key: *A*, 8.0 ml. $Al(OH)_3$ suspension; *B*, 8.0 ml. $Al(OH)_3$ plus magnesium trisilicate suspension; *C*, 1.0 Gm. magnesium trisilicate powder; *D*, two powdered tablets of $Al(OH)_3$ + magnesium trisilicate + mucin.

TABLE V.—*p*H OF MIXTURES OF 0.1 *N* HCl PLUS VARIOUS DOSES OF ANTACID

Time Min.	Buffer Capacity of Multiple Doses: *A*[a] (Reg. Dose = 8 Ml.) 4 Ml.	8 Ml.	12 Ml.	16 Ml.	*B*[a] (Reg. Dose = 8 Ml.) 4 Ml.	8 Ml.	12 Ml.	16 Ml.
1/2	1.45	1.81	1.90	2.30	3.20	4.00	5.00	5.40
5	2.34	3.62	3.98	4.28	3.95	4.60	5.70	6.07
10	3.93	4.05	4.24	4.35	4.07	4.78	5.97	6.27
15	4.15	4.21	4.30	4.38	4.13	4.94	6.05	6.35
20	4.20	4.25	4.36	4.42	4.15	5.03	6.14	6.45
30	4.22	4.28	4.38	4.51	4.20	5.14	6.23	6.54
60	2.11	4.15	4.24	4.33	3.90	4.20	4.50	5.03
90	1.63	3.74	4.14	4.24	3.45	4.06	4.23	4.28
120	1.54	2.50	3.82	4.18	2.42	3.90	4.15	4.20
150	1.26	1.89	2.94	3.93	1.98	3.68	4.07	4.13
180	1.23	1.64	2.20	3.54	1.78	3.42	3.97	4.10

[a] Key: *A*, aluminum hydroxide suspension; *B*, aluminum hydroxide plus magnesium trisilicate suspension.

DISCUSSION

Most of the nonsystemic antacids on the market can be divided into three groups:

1. Those containing principally aluminum hydroxide.
2. Those containing principally magnesium trisilicate.
3. Those which are combinations of aluminum hydroxide and magnesium trisilicate.

Group 1 gives a moderately rapid rise in *p*H, generally to 4.0 or 4.25. The fall in *p*H over the three-hour test period is gradual, remaining at about 3.5 for ninety minutes then declining to less than 3.0 at one hundred-eighty minutes.

Group 2 drives the *p*H immediately up to 5.0–7.0. The sustaining action is weak and the decline to below *p*H 3.0 is rapid.

Group 3 produces an initial rise in *p*H, with a subsequent decline, the speed of which depends upon the ratio of the active constituents present. A predominance of magnesium trisilicate furnishes its characteristic rapid rise in *p*H, generally above 4.5, but below that of antacids containing only magnesium trisilicate. The fall in *p*H is rapid, but not as fast as with preparations containing only magnesium trisilicate. When aluminum hydroxide is the major ingredient the rise in *p*H is not as rapid, but a near peak is sustained much better.

By comparison with groups 1 to 3 the resin antacid furnished a rapid rise in *p*H to almost neutrality, but its sustaining action was very weak, the *p*H falling to below 2.4 within ninety minutes.

Dihydroxy aluminum aminoacetate quickly elevated the *p*H to above 4.25. It also showed remarkable sustaining power, keeping the *p*H level above 3.65 for the entire three-hour test period.

In conclusion, it is worth noting that our preliminary experiments searching for a suitable general procedure demonstrated that an operating temperature of 25° and a medium of 0.1 *N* HCl gave results practically identical to those obtained using 37° and the artificial gastric juice of Toplis (4).

SUMMARY

1. A simple reproducible method was devised to compare the acid buffering capacities of 24 widely advertised antacids *in vitro*.

2. Aluminum hydroxide antacids are rather slow acting but their effect is prolonged.

3. Magnesium trisilicate antacids are rapid acting but their sustaining action is weak.

4. An antacid composed of a mixture of two parts of aluminum hydroxide and one part magnesium trisilicate seems to combine the best features of each.

5. Resinous antacids give a rapid rise almost to neutrality, but have a very weak sustaining power.

6. Dihydroxy aluminum aminoacetate gives both rapid action and prolonged effect.

7. Fresh milk exerts a buffering effect over a three-hour period *in vitro*.

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A New Spectrophotometric Method of Assay for Alexandria Senna Leaves (*Cassia Acutifolia*-Delile)*,†

By BERNARD V. CHRISTENSEN‡ and ISMAIL A. ABDEL-LATIF§

When Borntraeger's reaction is applied to Alexandria senna leaves, under controlled conditions, the color intensity of the ammonia layer was found to vary directly with the concentration of the extract or the quantity of drug. Spectrophotometrically, the extinction peak occurred at the wave length of 670 mμ The extinction varied directly with the concentration at this wave length. The method is rapid, accurate, and sensitive. It is applicable to all the emodin drugs. It should be used comparatively until a reference standard is established for every drug of the group.

THIS investigation was planned with a view to developing a new chemical method for the assay of the emodin-containing drugs.

The available methods of assay are quite unsatisfactory whether chemical, physical, or pharmacological. The pharmacopoeial requirements at the present time include nonspecific limit tests, identity tests, and some limits for water-soluble or alcohol-soluble extractives. In other words, no specific method for the assay of these drugs on the basis of their active ingredients has thus far been found satisfactory.

With these problems in mind, the idea occurred of trying to develop the common qualitative test called "Borntraeger's reaction" into a quantitative reaction and thus devise a method of assay for these drugs that might work on a comparative basis. With this as a basis, possibly a reference standard could later be established for each of these drugs.

EXPERIMENTAL

As a preliminary step, Borntraeger's reaction was carried out, using one sample of Alexandria senna. The extract obtained was completed to volume with ether so that each cc. of the ether extract represents 8 mg. of senna. From this stock ether extract, four equal volumes of different concentrations were quantitatively prepared. These were separately shaken with an equal volume of ammonia T. S. Table I summarizes the details. The intensity of the red colors, developed in the ammonia layers varied directly with the concentration of the ether extract used. These observations suggested further standardization and estimation of the color intensities spectrophotometrically.

Spectrophotometric Analysis.—The colors of the ammonia layers obtained were measured spectrophotometrically in accordance with the procedure described in a previous paper (1), except that standard 1-cm. light-path cuvettes were used as sample holders instead of test tubes. When transmission measurements were made above 650 mμ wave lengths, a red filter[1] was used in front of the entrance slit to remove the stray light from the second order spectrum.

A graph was made for every concentration plotting the extinction (E) values obtained against the wave lengths. Figure 1 shows the graphs as well as the correlation between the height of the extinction peak (at 670 mμ) and concentration of ether extract of the drug. Figure 2 shows the direct linear relationship that exists between the extinction values at 670 mμ wave length and the relative concentration. Table II summarizes the results obtained and shows that the error is negligible. The determination is very precise and sensitive in spite of all the factors involved that might influence the results, such as volatility of the ether and ammonia, consecutive extractions, experimental errors, and approximation in the conversion of transmission into extinction values.

Consequently, the spectrophotometric analysis of the ammonia layers can be used as a basis for a method of assay for senna.

Several series of experiments were carried out in order to study further the application of spectrophotometric measurements as a method of assay of anthraquinone-containing drugs, particularly Alexandria senna.

Various weights of one single sample of Alexandria senna were simultaneously used in a series of experiments.

The following weights of one single sample of Alexandria senna were used in a series of experiments:

Expt. No.:	I (Control)	II	III	IV	V
Senna weight:	10	9.5	5	4.5	2 Gm.

An ether extract was prepared in the same way as for the performance of the Borntraeger's reaction. All the samples were treated as if they were equal in weight. Each ether extract was completed to volume with ether so that 1 cc. of the ether extract (in the case of Experiment I or control) represents 10

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Presented to the Scientific Section, A. PH. A., Jacksonville meeting, April 30, 1949.

† Abstract of part of dissertation submitted to the graduate school by I. A. Abdel-Latif in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[1] The red filter used was compounded by the Corning Glass Co. The transmission characteristics of the red filter used in terms of % transmission measured against an air path are as follows:

Wave length, mμ:	600	620	640	660	670	680
% T:	0.4	7.8	73.5	86	86.5	87.0

mg. of the crude drug. Equal volumes of the various ether extracts were similarly shaken with equal volumes of Ammonia T. S. The colors developed in the ammonia layers varied directly in intensity with the concentrations of the ether extracts used or the quantity of the drug.

The colors of the ammonia layers were measured spectrophotometrically. Table III shows the results obtained. It can be seen from the table that the error is quite negligible in every case, except where only relative concentrations were as low as

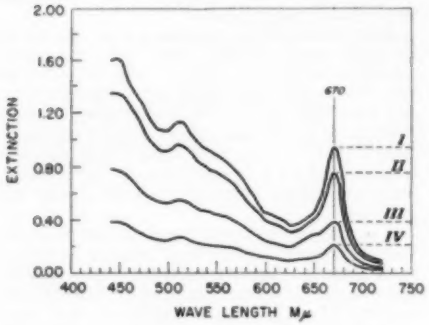

Fig. 1.—Spectrograph of Ammonia Layers of Various Concentrations.

Layer	Ether Extract,[a] cc.	Relative Concentrations Prepared, %	Found, %
I	25	100	100 (control)
II	20	80	80.593
III	10	40	40.008
IV	5	20	22.001

[a] One cc. ether extract = 8 mg. senna.

20% which probably would not exist without prominent adulteration that can be detected microscopically. Figure 3 shows the results graphically from which it can be concluded that the spectrophotometric measurements always showed the same relationship irrespective of the time interval at which the readings were made.

A second series of experiments was simultaneously carried out in order to test the sensitivity of the method. Table IV and Fig. 4 illustrate the results. It is again obvious that the method is very precise and sensitive at least to 1.5 mg. of the crude drug.

A third series of experiments was planned to check (1) whether or not the results could be duplicated, (2) whether or not color intensities are stable, and (3) to determine at what stages, if any, in the procedure, extracts could be left standing over night or longer for convenience of operation of the methods of assay. Six different weights of one sample of Alexandria senna leaves were used. The usual procedure was modified by storing tightly covered samples of the extract for varying periods to detect the effects of standing under refrigeration up to eighty hours. Table V shows results obtained when spectrophotometric measurements are read immediately after the ammonia layers are separated from the ether layers. Figure 5 illustrates graphs plotting extinction values at 670 mμ wave length against relative concentrations. Storage under refrigeration as well as other various conditions of checking are explained below the graph.

From the results obtained it was indicated that:

1. The results obtained can be exactly duplicated.

2. The measurements bear the same relationship regardless of:

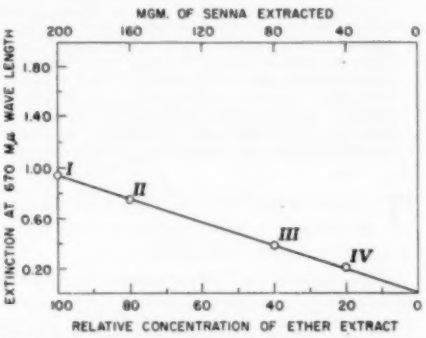

Fig. 2.—Spectrograph of Ammonia Layers of Various Concentrations.

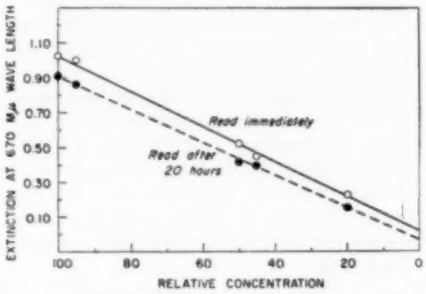

Fig. 3.—Spectrographs of Ammonia Layers Using Different Quantities of the Same Senna Sample.

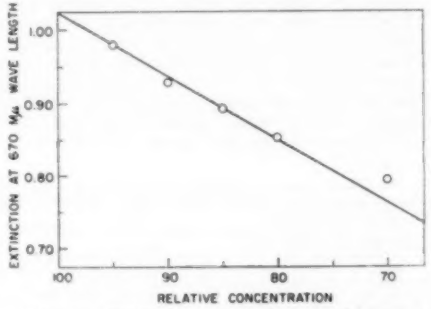

Fig. 4.—Spectrophotometric Analysis of Ammonia Layers Using Different Quantities of the Same Senna Sample.

TABLE I.—VARYING THE CONCENTRATION OF THE ETHER LAYER

Experiment No.:	I Control	II	III	IV	B Blank
I. Ether layer					
(*a*) Ether extract used, cc. (8 mg. senna/cc.)	25	20	10	5	0.0
(*b*) Pure ether added, cc.	00	5	15	20	25
Total volume of ether layers, cc.	25	25	25	25	25
Relative concentration of ether layer, %[a]	100	80	40	20	00
II. Ammonia layer					
Ammonia T. S. used, cc.	10	10	10	10	10
Color intensity of ammonia layer[a]	Very strong	Strong	Weak	Very weak	None
Relative intensity of colors[a] (approximately)	100	80	40	20	00

[a] Compare.

TABLE II.—RELATIONSHIP BETWEEN EXTINCTION VALUES AT 670 mμ OF THE AMMONIA LAYERS AND THEIR RELATIVE CONCENTRATIONS OR QUANTITIES OF SENNA EXTRACTED

Expt. No.	Ether Extract Used, Cc.	*E* at 670 mμ	Relative Concentration, %			Weight of Senna, Mg.		
			Prepared	Found	Error	Extract	Found	Difference
I Control	25	0.9405	100	100	0.000	200	200	0.000
II	20	0.758	80	80.593	0.741	160	161.086	1.086
III	10	0.377	40	40.008	0.020	80	80.016	0.016
IV	5	0.208	20	22.001	10.005	40	44.002	4.002

TABLE III.—EXTINCTION VALUES (AT 670 mμ) OF THE AMMONIA LAYERS VARY DIRECTLY WITH THE WEIGHT OF SENNA EXTRACTED

Expt. No.	Weight of Senna, Gm.	% *T*[a] Read Immediately				% *T*[a] Read After 24 Hours			
		E[b] at 670 mμ	Relative Concentration, %			*E*[b] at 670 mμ	Relative Concentration, %		
			Prepared	Found	Error		Prepared	Found	Error
I	10.0	1.022	100	100 (std.)	0.00	0.903	100	100 (std.)	0.00
II	9.5	1.000	95	97.75	2.81	0.854	95	94.5	0.53
III	5.0	0.520	50	50.89	1.80	0.411	50	45.5	9.00
IV	4.5	0.450	45	44.03	2.15	0.398	45	44.5	1.11
V	2.0	0.229	20	22.40	12.00	0.149	20	16.5	17.50

[a] % *T* = % transmission.
[b] *E* = Extinction.

TABLE IV.—SPECTROPHOTOMETRIC ASSAY OF VARIOUS QUANTITIES OF SENNA

Expt. No.	Weight of Senna, Gm.	% *T*[a] 670 mμ	*E*[b] at 670 mμ	Relative Concentration, %		
				Prepared	Found	Error
I	9.5	10.5	0.979	95	95.000	Control
II	9.0	11.8	0.929	90	90.147	0.181
III	8.5	12.8	0.893	85	86.621	1.907
IV	8.0	14.0	0.854	80	82.838	3.547
V	7.0	16.0	0.796	70	77.212	10.303

[a] % *T* = % transmission.
[b] *E* = Extinction.

TABLE V.—EXTINCTION VALUES (AT 670 mμ) OF THE AMMONIA LAYERS VARY DIRECTLY WITH THE WEIGHTS OF THE SENNA EXTRACTED

Expt. No.	Weight of Senna, Gm.	*E* at 670 mμ	% *T* Read Immediately — Relative Concentration, %		
			Prepared	Found	Error
I	10.0	1.553	100	100	Control
II	10.0	1.553	100	100	0.000
III	9.5	1.432	95	92.209	2.937
IV	8.0	1.260	80	81.133	1.416
V	7.5	1.222	75	78.586	4.781
VI	5.0	0.860	50	55.376	10.754

(*a*) The time interval between readings.
(*b*) Storage period under refrigeration.
(*c*) Kind of extract, whether alcoholic potassium hydroxide, ether, or ammonia extract was stored.

3. The assay procedure can be carried out at the convenience of the operator but all samples must be simultaneously and similarly treated.

The Proposed Procedure for the New Spectrophotometric Methods of Assay of Senna.—Due to the fact that this method of assay is a comparative method rather than a reference standard method, the procedure of the assay must be constant and definite in order to obtain dependable results.

PROCEDURE

Dry test sample of #60 senna powder at 70° over night or at 110° for three hours. Weigh accurately 10.0 Gm. of each sample of powder to be tested and place in an Erlenmeyer flask. Add 75 cc. of freshly prepared alcoholic potassium hydroxide (10% w./v.) to each 10-Gm. sample. Mix well with the powder and add glass beads to prevent bumping and then reflux for thirty minutes. Filter while warm with the aid of suction. Wash the flask and residue on the filter with about 15 cc. warm alcohol in two consecutive portions. Continue the suction for a few minutes to allow complete draining of filtrate

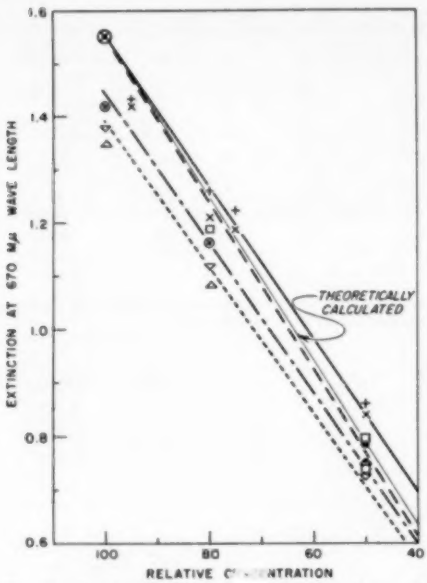

Fig. 5.—Spectrophctometric Analysis of Ammonia Layers under Various Conditions.
A, + —; B, ×; C, □--; D, ⁎; E, ⊙ —-; F, ○; G, ▽ —; H, Δ. The following coincide at ⊗: +, ×, □, and ⁎.

No.	Total Standing Time, Hr.	Extract Stored	Conditions
A	00.00	....	Read immediately
B	24.00	Ammonia of (*A*)	Read after 24 hr.
C	24	Alc. KOH ext. of (*A*)	Extracted with ether after acidification. The ether extract was shaken with ammonia T. S.
D	48	Ammonia of (*C*)	Read after 24 hr. more
E	24	Ether ext. of (*A*)	Shaken with ammonia T. S.
F	48	Ammonia of (*E*)	Read after 24 hr. more
G	48	Ether of (*C*)	Shaken with ammonia T. S.
H	80	Ammonia of (*G*)	Read after 32 hr. more

from the marc. Transfer the filtrate into a 100-cc. volumetric flask; wash the flask and funnel with a few cc. of alcohol and make up to volume with alcohol. (Each cc. of alc. KOH ext. = 100 mg. senna.) At this point, the extracts obtained can be left standing overnight in a refrigerator, on condition that all the samples are treated the same. Shake contents of the volumetric flask and then pipette 25 cc. into a 250-cc. separatory funnel (25 cc. alc. KOH ext. is equivalent to 2.5 Gm. of the crude drug senna). Add 25 cc. distilled water to the alcoholic potassium hydroxide extract and 10 cc. (or more) of dilute hydrochloric acid, to make the *p*H of the mixture about 2.

Extraction with Ether.—Shake with ether by usual procedure using 30-cc. and then five 20-cc. portions. Collect all the ether extracts and wash the total ether extract with a mixture of 5 cc. of dilute hydrochloric acid and 10 cc. of distilled water. Shake aqueous washings with 15 cc. of ether. Add to the total ether extract. Transfer all the ether extracts to a 200-cc. volumetric flask and make up to volume with ether. (Each cc. of ether extract = 12.5 mg. crude drug.) At this stage, the experiment can be left standing over night in a refrigerator with all containers well closed.

Shaking Out with Ammonia.—Measure 30 cc. of the ether extract into a separatory funnel. Add 10 cc. of freshly prepared ammonia T. S. Shake gently, let stand, then shake vigorously and centrifuge for about five minutes at 5000 r. p. m. or until the ammonia layer separates completely and sharply from the ether layer. At this stage, if the spectrophotometric measurements cannot be immediately made, the ammonia layer can be well stoppered and refrigerated overnight. Again it should be emphasized that all the samples must be similarly treated. Prepare a blank by shaking 30 cc. of pure ether with 10 cc. ammonia T. S. Take the separated ammonia layer and use it as the blank.

Spectrophotometric Analysis.—Determine the % transmission measurements of the ammonia layer at 670 mμ wave length. From the % transmission values obtained, determine their corresponding extinction values from the special conversion tables. The extinction varies *directly* with the relative concentration of the anthraquinone derivatives. From this direct quantitative linear relationship the unknown concentrations should be interpreted from the extinction values of their ammonia layers.

Results can be interpreted either graphically by plotting the necessary graphs or mathematically, and confirmed by both means.

DISCUSSION

Explanation of the various steps followed in the procedure proposed:

1. All of the samples to be assayed must be treated in an exactly similar manner and the procedure carried out on all of them simultaneously in order to get comparative results.

2. The drying of the powder is intended to level the moisture contents of the samples. It is not performed to get total dryness of the powders.

3. The alcoholic potassium hydroxide (10%) must be freshly prepared.

4. The alkali hydrolysis is preferred to the acid hydrolysis since the alkali keeps the anthraquinones in solution while the acid precipitates them. The alkaline extract can be left standing without danger of precipitation (1).

5. Water is added to the alkaline extract immediately before the extraction with ether to favor the extraction process.

6. The anthraquinone derivatives are thrown out of the solution as a light green precipitate by acidification. This precipitate is readily and completely extracted by the ether.

7. Ether apparently is the best organic solvent to use, as shown by the investigation carried out in a previous paper (1).

8. The washing of the ether extract with the acidulated water was found advantageous by removing the greenish tint from the ether extract but will not remove any of the anthraquinones. The subsequent shaking of the acidic aqueous washings with ether is a precaution to extract the traces of the ether extract that may go into solution in the aqueous layer of washings.

9. It is recommended to use a volume of the ether extract approximately three times the volume of ammonia layer required in order to prevent or decrease formation of emulsions

10. The quantities of the ammonia layer and of the ether extract may be adjusted to the convenience of the operator.

11. Ammonia T. S. should be always freshly prepared to assure even strength in every assay.

12. The ammonia layer should be separated from emulsions with an ether layer by centrifuge to save time and prevent changes in colors.

SUMMARY

A new spectrophotometric method of assay for Alexandria senna leaves has been devised. The method is rapid, accurate, and sensitive. The method has been applied to six different lots of U. S. P. Alexandria senna leaves and they showed varying quantities of anthraquinone derivatives.

It is quite possible that the method could be applied to all the other emodin drugs. Until Reference Standards are established for each of these drugs, the method can be applied comparatively.

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Dichloro Aluminum Aminoacetate, a New Anti-Perspirant and Deodorant*

By I. W. GROTE, J. M. HOLBERT, and PEGGY WALKER CROSS

Although aluminum chloride is undoubtedly the best underarm deodorant and antiperspirant, it suffers from the disadvantage that it readily hydrolyzes to yield free hydrochloric acid which will cause tenderization of fabrics and skin irritation. The addition of buffering agents, such as urea, helps control free acidity but these buffers may be more readily removed from the skin by moisture than the aluminum salt. We have prepared the readily soluble salt dichloro aluminum aminoacetate which possesses an internal buffer in the NH_2 group. It shows some promise as an effective deodorant.

THERE are three requirements for an effective underarm deodorant. First, the compound used must be an astringent, which will serve to block, partially or completely, the secreting gland and thus inhibit the formation of perspiration. Second, the compound must be antiseptic in nature and thus prevent putrefaction of perspiration with the formation of disagreeable odors, and third, the compound must be nonirritating under normal conditions of use (1–3). Astringency is promoted by a lowering of *p*H; however at a *p*H much below 3.0 there is apt to be tenderizing of the clothing and irritation of the skin.

The most satisfactory and widely used astringents are aluminum sulfate and aluminum chloride, both being markedly astringent, reasonably harmless to the skin in effective concentration, and adaptable to a wide variety of preparations. A serious drawback, however, is their acidity, which increases with hydrolysis.

Many buffers and inhibitors, such as urea, oxides, and related compounds, have been tried to prevent rotting of fabrics which takes place when the acid salts of aluminum are used. Although these inhibitors are effective, in many cases the astringent chemical is selectively absorbed and retained in the clothing in an unbuffered form. Thus the use of a hot iron releases free acid from the fabric and quickly deteriorates the fibers.

If the buffer could be made an inherent part of the astringent molecule, however, this separation of inhibitor and astringent could not take place and any acid formed during hydrolysis would be neutralized by the buffer present in the compound itself.

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EXPERIMENTAL

In an attempt to prepare dihydroxy aluminum aminoacetate by a new method, aluminum chloride and sodium glycinate were mixed in concentrated aqueous solution.

On warming the solution slightly, the entire mixture solidified to a jelly-like consistency, having no resemblance to the compound sought.

The possibility appeared that this jelly-like substance might have unique properties as a deodorant, by virtue of its relation to aluminum chloride. Upon investigation, the jelly was found to have a *p*H of somewhat above 3.0, would not rot cloth, and was nonirritant when applied to sensitive areas of the body. This jelly-like material, which undergoes syneresis upon prolonged standing, was therefore further investigated.

The solution upon concentration deposited sodium chloride. Further investigation showed the reaction is as follows: $AlCl_3 + NH_2CH_2COONa \rightarrow NH_2CH_2COOAlCl_2 + NaCl$.

The removal of the salt formed proved most difficult but was partially accomplished as follows: 100 cc. of a solution of sodium glycinate and aluminum chloride, in correct molecular proportions, was placed in a collodian bag and dialyzed against running water until the dialysate was free from chloride ion. The contents of the bag were dried under vacuum at a temperature below 50°. The dried material when redissolved in water had a *p*H of 3.5, and was soluble approximately 1 Gm. in 1 cc. of distilled water. The dried material is relatively nonhygroscopic, but analysis of the dried material gave the following results:

	Found, %	Calculated for $NH_2CH_2COOAlCl_2 \cdot 6H_2O$, %
Chlorine	30.95	25.36
Nitrogen	4.80	5.00

The sample thus appeared to have some sodium chloride still present. A further attempt to remove salt by selective precipitation with successive additions of ethanol gave a product with the same properties but again containing salt. A second method of preparation was therefore tried.

The preparation of dichloro aluminum aminoacetate by the reaction of dihydroxy aminoacetate and concentrated hydrochloric acid.—Seventy-six and four-tenths grams of dihydroxy aluminum aminoacetate containing 11.6% moisture was slowly added to 83 cc. of concentrated hydrochloric acid. The reaction was quite exothermic and external cooling was necessary. On standing overnight the material formed a thick gel which was evaporated at low temperature under vacuum. Analysis of the dried material gave the following results:

	Found, %	Calculated for $NH_2CH_2COOAlCl_2 \cdot 6H_2O$, %
Chlorine	28.32	25.36
Nitrogen	5.25	5.00
Aluminum	10.04	9.64

Inspection of these results, as well as those previously obtained, suggested that the compound might be dichloro aluminum aminoacetate hexahydrate. Calculation shows the results for chlorine, nitrogen, and aluminum correspond to a ratio of 2.12, 1.01, and 1.00, a reasonably close agreement with the theoretical value of 2:1:1.

Taking advantage of the fact that aluminum chloride is quite soluble in absolute alcohol, the following experiments were carried out to demonstrate that the reaction product was actually a compound and not a mixture of aluminum chloride buffered with sodium glycinate.

Forty grams of anhydrous aluminum chloride and 49.65 Gm. of glycine were dissolved in a small amount of water; the *p*H of the resulting solution was 1.5. Since it proved impossible to evaporate this solution to a solid, a weighed sample of the resulting thick syrup was placed in a Soxhlet thimble and extracted for three hours with absolute alcohol. During this period a total of 45.5% of the aluminum chloride had been extracted.

A sample of the reaction product from sodium glycinate and aluminum chloride, prepared as previously described, when subjected to the same extraction with absolute alcohol lost 1.7% of the sample weight. To demonstrate further that aluminum chloride, if present as such in the product, would have been extracted by this procedure, a mixture of equal weights of aluminum chloride and the reaction product above was similarly extracted with absolute alcohol. A total of 86% of the aluminum chloride added was extracted by the ethanol. Thus on the basis of analysis, physical behavior, and the probable result of the reaction, the structure $NH_2CH_2COOAlCl_2 \cdot 6H_2O$, is suggested for the compound.

Dichloro aluminum aminoacetate has been successfully compounded with various cosmetic bases and has proved to be an effective underarm antiperspirant and deodorant. Such preparations have been used by approximately twenty people over a period of three years. There has been no evidence so far that such preparations tend to damage fabric or cause local skin irritation except in the case of one individual who appears to be sensitive to all aluminum-containing compounds. This preliminary study shows the compound worthy of more detailed investigation with regard to skin irritation, effectiveness, and possible damage to fabric.

SUMMARY

The reaction between aluminum chloride and sodium glycinate has been investigated and evidence is presented indicating the principal reaction product to be a hydrate of dichloro aluminum aminoacetate, $NH_2CH_2COOAlCl_2$. This compound when compounded with various cosmetic bases, has proved to be an effective underarm antiperspirant and deodorant worthy of further study.

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A Study of Antacid Buffers. III. The Antacid Properties of Some New Aluminum Glutamates*

By J. W. LEMAISTRE, J. M. HOLBERT, and I. W. GROTE

Acute toxicity studies were made with mice on tetrahydroxydialuminum magnesium diglutamate (compound II) and tetrahydroxydialuminum glutamate (compound III). They showed no toxic effects in doses up to 50 Gm. per kilogram of body weight. Studies of tetrahydroxydialuminum magnesium diglutamate and tetrahydroxydialuminum glutamate are now in progress in a large clinic. Preliminary reports of the first three months' experience indicate that the antacid properties of the two compounds in cases of gastric hyperacidity are in agreement with the expectations from the results of acid-neutralizing tests here reported.

OUR EARLIER reports (1, 2) were concerned with the antacid properties of dihydroxyaluminum aminoacetate (3). The excellence of that substance as an antacid prompted the extension of the work to include the basic aluminum salts of other amino acids. We therefore sought to prepare and evaluate as antacids the following salts of the dibasic amino acid, glutamic acid: dihydroxyaluminum sodium glutamate (I), tetrahydroxydialuminum magnesium diglutamate (II) and tetrahydroxydialuminum glutamate (III). In this series one carboxyl group of the glutamic acid is subject to salt formation with the dihydroxyaluminum group, the other with sodium, magnesium, and the dihydroxyaluminum group, respectively.

The first two of these substances were made by reaction of aluminum isopropylate with aqueous solutions of sodium acid glutamate in one case and magnesium acid glutamate in the other. The third was made by reaction of two moles of aluminum isopropylate with one of glutamic acid. The products were obtained as white solids which were insoluble in water. They were not entirely pure, analyses indicating the presence of 10–15 per cent of hydrous aluminum oxide. That salt formation occurred as anticipated is evidenced by the fact that sodium and magnesium glutamates did not appear to be leached from the sodium and magnesium compounds by cold water and that all three products precipitated as granular, easily filtered materials quite unlike aluminum hydroxide.

Since the acid-neutralizing properties of the three products were found reproducible from batch to batch, they were evaluated as antacids without further purification. The rapidity of neutralization of artificial gastric juice was determined by the method of Holbert, Noble, and Grote (1) with results which are shown in Table I and Fig. 1. The data show that the rapidity of

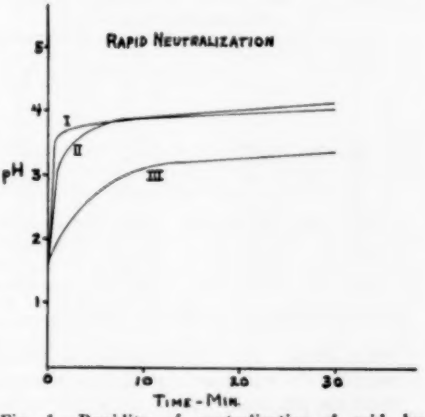

Fig. 1.—Rapidity of neutralization of acid by antacid buffers.

Curve I, dihydroxyaluminum sodium glutamate; curve II, tetrahydroxydialuminum magnesium diglutamate; curve III, tetrahydroxydialuminum glutamate.

neutralization decreases in this order: sodium-aluminum salt, magnesium-aluminum salt, dialuminum salt. The rate at which I and II neutralize acid in this test (in which the acid is present in excess for the whole period) is very large, exceeding that previously reported for dihydroxyaluminum aminoacetate (1). The rate of neutralization by III is somewhat less than that of dihydroxyaluminum aminoacetate. All three of the new products appear to be in an acceptable range for antacids.

The prolonged buffering action of these compounds was studied by the method of Johnson and Duncan (4) as modified by Holbert, Noble, and Grote (2). In this method the antacid is ini-

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TABLE I.—RAPIDITY OF NEUTRALIZATION OF ACIDITY BY ANTACID MATERIALS[a]

Time, Min.	pH I	pH II	pH III
0	1.63	1.64	1.64
1	3.58	3.11	1.90
3	3.71	3.61	2.45
5	3.81	3.78	2.72
10	3.90	3.90	3.09
15	3.95	3.99	3.20
20	4.00	4.01	3.28
25	4.00	4.04	3.38
30	4.03	4.08	3.40

[a] The compounds are numbered I, II, III as in the text.

tially present in excess over the acid and as the test proceeds, the amount of antacid is systematically decreased and the amount of acid increased. The results appear in Table II and Fig. 2. All three compounds exhibited significant

TABLE II.—PROLONGED BUFFERING ACTION OF ANTACID MATERIALS[a]

Time, Min.	pH I	pH II	pH III
0	1.62	1.59	1.59
1	4.10	3.79	3.05
3	4.30	4.11	3.49
5	4.35	4.21	3.59
10	4.41	4.34	3.74
20	4.29	4.14	3.80
30	4.17	4.07	3.81
40	4.02	4.05	3.76
60	3.85	3.96	3.68
80	3.70	3.79	3.60
100	3.52	3.68	3.47
120	3.33	3.57	3.30

[a] The compounds are numbered I, II, III as in the text.

buffer action. The compounds I and II raised the *p*H of the test system rapidly to 4.2–4.4 after which the *p*H slowly receded as further acid was added. In both cases the *p*H remained above 3.2 for over two hours. Compound III raised the *p*H to a maximum value of 3.8 and maintained the *p*H above 3.2 for over two hours. The data for the latter material closely resemble those previously reported for dihydroxyaluminum aminoacetate (2). With respect to prolonged buffering action all three of the new products appear to be satisfactory for use as antacids.

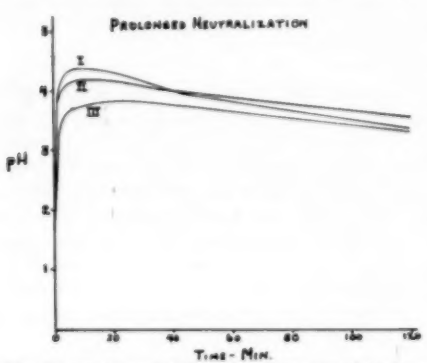

Fig. 2.—Prolonged buffer action of antacid buffers. Curve I, dihydroxyaluminum sodium glutamate; curve II, tetrahydroxydialuminum magnesium diglutamate; curve III, tetrahydroxydialuminum glutamate.

EXPERIMENTAL

Dihydroxyaluminum Sodium Glutamate (I).—Monosodium glutamate (16.9 Gm., 0.1 mole) was dissolved in 100 ml. of water and stirred vigorously while adding 20.4 Gm. (0.1 mole) of aluminum isopropylate. Heat was evolved and precipitation was rapid. The mixture was filtered after cooling, and the solid dried at 100°. The product was a white solid which was insoluble in water, 18 Gm. Analysis indicated considerable coprecipitation of alumina.

Anal.—Calcd. for $C_5H_8O_4NAlNa$: N, 6.1%; Al, 11.7%. Found: N, 4.1%; Al, 15.9%.

Tetrahydroxydialuminum Magnesium Diglutamate (II).—Glutamic acid (29.4 Gm., 0.2 mole) and 4.0 Gm. (0.1 mole) of magnesium oxide were added to 100 ml. of water and warmed to 50°. When the solution became clear it was stirred vigorously while adding 40.8 Gm. (0.2 mole) of aluminum isopropylate. Heat was evolved and precipitation began at once. After cooling the solid was filtered off and dried at 100°. The product was a white, granular solid which was insoluble in water, 41 Gm.

Anal. Calcd. for $C_{10}H_{18}O_{12}N_2Al_2Mg$: N, 6.4%; Al, 12.4%. Found: N, 4.4%; Al, 13.1%.

Tetrahydroxydialuminum Glutamate (III).—Glutamic acid (14.7 Gm., 0.1 mole) was dissolved in 150 ml. of boiling water. This solution and 40.8 Gm (0.2 mole) of aluminum isopropylate were separately and simultaneously poured into 100 ml. of well-stirred isopropyl alcohol. Precipitation was immediate. After the mixture cooled, the product was filtered off and dried at 100°. It was a white, granular solid which was insoluble in water, 26 Gm.

Anal.—Calcd. for $C_5H_{11}O_4NAl_2$; N, 5.2%; Al, 20.2%. Found: N, 4.7%; Al, 19.7%.

Rapidity of Neutralization of Artificial Gastric Juice.—The procedure has been described in detail previously (1). One gram of the powdered material under test was added to 150 ml. of artificial gastric juice (5) and well stirred at 37.5° in a thermostat. The *p*H was measured at intervals by means of a Beckman *p*H meter. The results appear in Table I and Fig. 1.

Prolonged Buffering Action.—The procedure has been described in detail previously (2). Two grams of the powdered material under test were added to 150 ml. of artificial gastric juice (5) and well stirred at 37.5° in a thermostat. At the end of ten minutes and after every ten-minute period thereafter, 20 ml. of the test mixture was removed and replaced by 20 ml. of fresh artificial gastric juice. The *p*H was determined at intervals by means of a Beckman *p*H meter. The results appear in Table II and Fig. 2.

SUMMARY

1. Three new basic aluminum glutamates were prepared and evaluated as antacids. They are dihydroxyaluminum sodium glutamate, tetrahydroxydialuminum magnesium diglutamate, and tetrahydroxydialuminum glutamate.

2. All three materials showed rapid neutralization of the acidity of artificial gastric juice as well as prolonged buffering action. They appear suitable for use as antacid buffers.

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Studies in the Hydrophylic Properties of Possible Ointment Base Constituents. V. The Isomeric Eicosanols*

By ALFRED HALPERN† and JOHN G. ADAMS‡

A series of isomeric eicosanols were synthesized. The water numbers of these compounds were determined as well as their effect on the surface and interfacial tensions of a mineral oil-water system. The 1-, 2-, and 4-eicosanols exhibit some desirable properties that merit further investigation as to their use in ointment bases. The 6-, 8-, and 10-eicosanols showed little, if any, emulsifying properties.

RECENTLY it was shown (1) that *n*-eicosanol-1 caused the greatest potentiation of the water number of petrolatum, of the series of fatty alcohols studied. A search of the literature revealed that the secondary higher fatty alcohols had received little attention. In fact, many of the isomers of the common ointment base ingredients (i.e., lauryl, myristyl, cetyl, and stearyl alcohols) are unknown. It was deemed of interest, therefore, to prepare an isomeric series of the higher secondary alcohols and to evaluate them as possible ointment base ingredients.

EXPERIMENTAL

In view of the desirable properties of *n*-eicosanol-1, a series of isomeric secondary eicosanols were chosen for study. The only *n*-eicosanols reported in the literature are the *n*-eicosanol-1, (2–5), which has received some attention, and *n*-eicosanol-2, (6). The secondary alcohols used in this study were prepared, in fairly good yields, through the action of the appropriate Grignard reagent on an aldehyde. The compounds were purified through repeated crystallization from ethanol-benzene mixtures.

Preparation of the Isomeric Eicosanols.—To a three-necked flask fitted with a reflux condenser, stirrer, and inlet tube containing 0.1 mole of the appropriate alkyl magnesium bromide in ether solution, was added a 0.15 mole solution of the aldehyde in dry ether. When all the aldehyde had been added, the mixture was refluxed for two hours and decomposed with acid-ice water. The ether layer was separated, washed twice with a 5% hydrochloric acid solution and once with a 10% sodium bicarbonate solution, and dried over anhydrous sodium sulfate. The ether was distilled under reduced pressure and the solid residue crystallized from alcohol-benzene mixture. The physical properties of the starting materials, the resultant products, and yields are presented in Table I.

n-Eicosanol-1 was prepared in a similar manner from stearyl magnesium bromide and ethylene oxide (1). The product was worked up in the manner described above (Table I).

The water numbers of the experimental compounds were determined in the manner described by Halpern and Zopf (7) and are reported in Table II.

Since most of these compounds were prepared for the first time, the effects of the experimental compounds on the surface and interfacial tension of systems comparable to those encountered in ointments were studied. The tensiometric measurements were determined with the Cenco Du Nouy interface tensiometer, using a ring with a ratio of R/r of 53. The usual corrections for the ring method were applied to the observed data (8). Gel formation was noted with the oil solutions of the eicosanols at room temperature, and the interfacial tensions were determined at 60°, at which temperature complete solution was affected. The data presented in Table III

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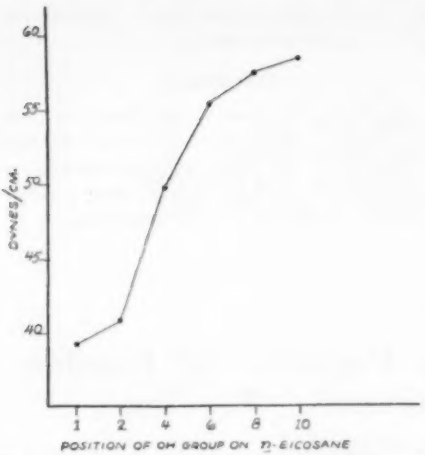

Fig. 1.—The surface tension of a saturated aqueous solution of the isomeric eicosanols at 25°.

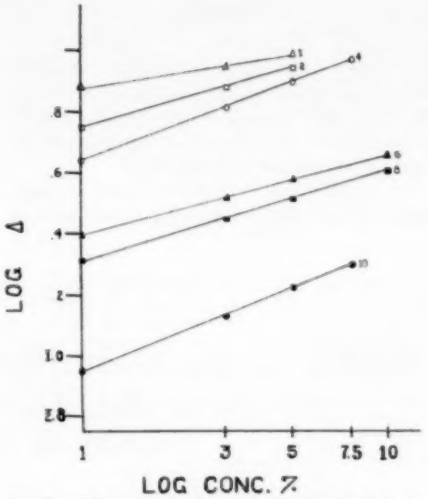

Fig. 2.—Effect of isomeric eicosanols on the interfacial tension at 60°

TABLE I.—THE PREPARATION OF THE ISOMERIC SECONDARY EICOSANOLS

Alkyl Bromide[a]	B. P.	Aldehyde	n-Eicosanol	M. P.	Yield, Gm. Found	Yield, Gm. Theory	Yield, %
Stearyl bromide	214–218/15 mm.	[b]	n-Eicosanol-1	67–69	18.0	29.6	61
Stearyl bromide	214–218/15 mm.	Acetaldehyde	n-Eicosanol-2	58–59	11.8	29.6	40
Cetyl bromide	194–196/16 mm.	Butyraldehyde	n-Eicosanol-4	43–44	13.3	29.6	45
Myristyl bromide	170–172/25 mm.	Hexylaldehyde[c]	n-Eicosanol-6	54–56	9.9	29.6	33
Lauryl bromide	175–180/45 mm.	Octylaldehyde[c]	n-Eicosanol-8	52–54	16.9	29.6	57
Decyl iodide	132–133/15 mm.	Decylaldehyde[c]	n-Eicosanol-10	65.5	17.8	29.6	60

[a] Prepared in our laboratory according to the procedure described in Organic Synthesis, Vol. I., p. 29(1941).
[b] Ethylene oxide was used to prepare n-Eicosanol-1.
[c] These aldehydes were supplied to us by Fritzche Bros., Inc., New York, N. Y., through the courtesy of Fred C. Hilbert and T. J. Coyle.

TABLE II.—WATER NUMBERS OF THE ISOMERIC EICOSANOLS IN PETROLATUM[a]

Eicosanol	Concentration, % 1	2	3	4	5	7.5	10
1	30.3	38.1	43.1	51.2	54.3	40.3	30.0
2	24.1	28.2	34.3	40.1	44.2	37.1	36.0
4	20.4	24.3	30.3	32.4	36.6	36.2	25.1
6	14.1	14.3	16.2	17.1	18.6	18.0	14.0
8	12.2	14.2	16.0	16.8	17.6	17.4	17.0
10	12.0	13.8	15.0	16.8	17.4	17.4	16.8

[a] The water number of the petrolatum used was 10.2.

and Figs. 1 and 2 are the means of eight observations. The relative effects of the experimental compounds were calculated from the equation (9) where γ_{IFo} is the interfacial tension of the original

$$\Delta_{IF} = \frac{\gamma_{IFo} - \gamma_{IF}}{\gamma_{IFo}} = K_{IF}C^{1/n}$$

system, γ_{IF} equals interfacial tension of the system containing the experimental compound at concentration C, K_{IF} and n being constants. By converting the above equation to its logarithmic form and

TABLE III.—THE INTERFACIAL TENSION OF THE EICOSANOL-MINERAL OIL-WATER SYSTEM[a]

n-Eicosanol	Dynes/Cm. Concentration, % 1	3	5	7.5	10	Δ_{IF} Concentration, % 1	3	5	7.5	10
1	8.3	4.2	1.9	...	...	0.74	0.87	0.94	...	...
2	12.8	8.3	4.2	...	...	0.60	0.74	0.82	...	...
4	18.6	11.2	6.8	2.9	...	0.42	0.65	0.79	0.91	...
6	24.0	21.7	20.2	...	17.3	0.25	0.33	0.37	...	0.46
8	25.3	23.1	19.6	...	19.2	0.21	0.38	0.39	...	0.40
10	29.5	27.6	27.9	25.6	...	0.08	0.14	0.16	0.20	...

[a] The temperature of the system was 60°.

plotting the log Δ_{IF} vs. log concentration an approximately rectilineal curve is obtained.

$$\log \Delta_{IF} = \log K_{IF} + 1/n \log C$$

The effect of the 2-, the 4-, and the 6-eicosanol on the antiseptic activity of various agents as evidenced by the agar-plate method was determined in the usual manner (10). No appreciable difference was noted with these compounds as compared with the previously reported activity of the fatty alcohols (10).

DISCUSSION

The secondary *n*-eicosanols were not as efficient emulsifying agents when compared with the primary alcohol. The 2- and 4-eicosanols have some desirable properties that merit further investigation; the 6-, 8-, and 10-alcohols showed very little activity. It is of interest to note that the greater the steric hindrance to the hydrophylic group the less the emulsifying efficiency. The gel formation of the alcohol-oil solutions, at room temperature indicates the possibility that these agents act in a manner described by Pink (11) for the mechanism of emulsification by a solid mutually insoluble in both phases. It is obvious from the complexities of the emulsification process that more than one factor contributes to its formation.

SUMMARY

1. A series of isomeric eicosanols were synthesized and investigated for possible use in ointment bases.

2. The water numbers of these compounds were determined as well as their effect on the surface and interfacial tensions of a mineral oil-water system.

3. The 1-, 2-, and 4-eicosanols exhibit some desirable properties that merit further investigation as to their use in ointment bases. The 6-, 8-, and 10-eicosanols showed little, if any, emulsifying properties.

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Certain Substituted Thiobisacetimides and Corresponding Sulfones*

By PAUL R. RASANEN†, ‡ and GLENN L. JENKINS

Hypnotics and anticonvulsants such as barbiturates, hydantoinates, monoureides, and oxazolidiones all contain the functional group RR′C(—CONH—, where R represents an alkyl or aryl group. The purpose of this work was to attempt to prepare a heterocyclic compound containing sulfur as well as the functional group so characteristic of hypnotics and anticonvulsants. Thiobisacetimide [$S(CH_2CO)_2NH$] was prepared as the parent heterocycle and the syntheses of the following alkyl and aryl substituted derivatives and intermediates are described: (*a*) N-phenylthiobisacetimide, (*b*) N-benzoylthiobisacetimide, (*c*) sulfonylbisacetimide, (*d*) N-phenylsulfonylbisacetimide, (*e*) α,α′-thiobispropionimide, (*f*) α,α′-thiobis-*n*-butyrimide, (*g*) N-phenyl-α,α′-thiobis-*n*-butyrimide, (*h*) α,α′-thiobisisobutyrimide, (*i*) α,α′-sulfonylbisisobutyrimide, (*j*) N-methyl-α,α′-sulfonylbisisobutyrimide, and (*k*) N-methyl-α,α′-thiobisisobutyrimide. A total of seventeen compounds is reported.

A CLOSE study of the organic medicinal products which possess sedative and hypnotic activity reveals a striking similarity in their structure. The barbiturates, hydantoinates, monoureides, and amides all contain the functional group RR′C(—CONH—. Even the disulfones of substituted methane, such as tetronal, possess the dialkyl-substituted methylene group, but in this case the carbonyl group has been replaced by a sulfonyl group. The purpose of this work was to attempt to prepare some heterocyclic compounds containing sulfur as well as the group so characteristic of hypnotics and anticonvulsants.

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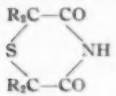
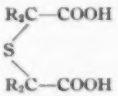
This investigation consists of attempts to prepare various alkyl-substituted thiobisacetimides (I) and corresponding sulfones.



(I)



(II)

This heterocycle is characterized not only by the functional group so characteristic of hypnotics, but also by a sulfur atom and an additional

group. The parent heterocycle (thiobisacetimide) was first prepared by Schulze (1) in 1866, but no other data on this series of compounds have been reported in the literature.

The cyclic imides of the various thio compounds were prepared by heating the diammonium salt of the corresponding dicarboxylic acid (II). This procedure could not be used in the case of the corresponding sulfonyl compounds, because heat caused the dicarboxylic acid to lose two molecules of carbon dioxide. In this case the corresponding anhydrides were prepared which were converted to the anilic or amic acids and attempts were made to cyclize these with very little success. Attempts to prepare the cyclic sulfonyl compounds from the corresponding thio compounds by oxidation with hydrogen peroxide at room temperature were not too successful. α,α'-Sulfonylbisisobutyrimide was prepared in good yields by this procedure in a few instances, but generally a compound of unknown structure resulted. This, however, could be readily converted to the cyclic imide by neutralizing with sodium hydroxide using phenolphthalein as an indicator and subsequent acidification with hydrochloric acid. Attempts to prepare α,α'-sulfonylbispropionimide and α,α'-sulfonylbis-*n*-butyrimide were unsuccessful. Treating thiobisacetimide with hydrogen peroxide resulted in a small amount of product, the analysis of which compares favorably with the corresponding sulfone. However, in this case also, decomposition was in evidence.

The N-methyl derivative of α,α'-sulfonylbisisobutyrimide was obtained in crystalline form by treating the cyclic imide with dimethylsulfate in the presence of sodium hydroxide. Similar treatment of α,α'-thiobisisobutyrimide resulted in a liquid derivative with a characteristic camphoraceous odor.

The thiobiscarboxylic acids were prepared by treating the sodium salts of the α-halogenated acids with sodium sulfide and the corresponding sulfonyl compounds were prepared by oxidation with potassium permanganate. For the preparation of α,α'-thiobisisobutyric acid a special procedure was necessary since α-bromoisobutyric acid has a tertiary bromine atom which is very unstable. In this case ethyl α-bromoisobutyrate was refluxed under anhydrous conditions with anhydrous sodium sulfide prepared by passing hydrogen sulfide through an alcoholic solution of sodium ethoxide. A similar procedure failed to produce α,α'-thiobis(diethylacetic) acid.

Cis-trans isomerism is possible with α,α'-thiobisbutyrimide and α,α'-thiobispropionimide because of the two asymmetric carbon atoms in each molecule. No exhaustive attempts were made to isolate or identify the various isomers but in two instances two compounds with different melting points, but of apparently the same composition, were obtained.

EXPERIMENTAL

1. Preparation of the Thiobiscarboxylic Acids.—For the preparation of thiobisacetic acid a procedure used by Loven (4) gave the best results, while the other thiobiscarboxylic acids were best prepared by a method proposed by Loven (2) and Ahlberg (3).

2. Preparation of the Diammonium Thiobiscarboxylates.—This procedure was generally used for the preparation of all the diammonium salts of the corresponding thiobiscarboxylic acids. The acid was dissolved in absolute ethyl alcohol and this alcoholic solution was saturated with ammonia gas. The mixture, which already had a crystalline precipitate, was cooled in an ice-salt bath and filtered. Concentration of the alcoholic filtrate and subsequent cooling gave increased yields of the diammonium salt. The yields approximated 80–90% of the theoretical.

3. Preparation of Thiobisacetimide.—This procedure was essentially that used by Schulze (1) and was used throughout for the preparation of the cyclic imides with modifications as needed in temperature and crystallizing solvents.

Diammonium thiobisacetate was heated in a suitable flask in an oil bath between 200–220° for about one hour. The resulting oil was recrystallized several times from hot water. The long, needle crystals melted at 128–129° (reported 129°). The yield was 50% of the theoretical.

4. Synthesis of N-Phenylthiobisacetimide: *Preparation of Thiobisacetic Anhydride*—Thiobisacetic acid was treated with an excess acetyl chloride at reflux temperature according to the method of Anschutz and Biernaux (5). The yield was 59% of the theoretical; m. p. 100° (reported 102°).

Thiobisacetanilic Acid.—The anhydride obtained above was treated with aniline according to the method used by Anschutz and Biernaux (5). The yield was 83% of the theoretical; m. p. 100° (reported 102°).

N-Phenylthiobisacetimide.—Refluxing 14 Gm. of thiobisacetanilic acid with an excess acetyl chloride for three hours resulted, after cooling in an ice bath, in a crystalline product which was recrystallized three times from glacial acetic acid. The yield was 8

Gm. (62%); m. p. 211–212° (dec.) (corrected).

Anal.—Calcd. for $C_{10}H_9O_2NS$: N, 6.76; S, 15.46. Found: N, 6.69; S, 15.94.

5. Preparation of N-Benzoylthiobisacetimide.—Two and one-half grams of thiobisacetimide was dissolved in 4.5 cc. of pyridine and the mixture gradually treated with 3 Gm. of benzoyl chloride, according to the method used by Titherley for the preparation of N-benzoylsuccinimide (6). After twelve hours a product separated which was recrystallized from alcohol. The yield was 1.6 Gm. (34%); m. p. 153° (corrected).

Anal.—Calcd. for $C_{11}H_9O_2NS$: N, 5.96; S, 13.62. Found: N, 5.86; S, 14.14.

6. Synthesis of N-Phenylsulfonylbisacetimide: *Preparation of Sulfonylbisacetic Acid.*—This acid was prepared by the method offered by Alden and Houston (7) except that the final product was isolated by extraction with ethyl acetate rather than by crystallization from the original acidified solution. Yields approximated 60% of the theoretical; m. p. 184–186° (corrected) [reported 182° (2) and 184° (7)].

Sulfonylbisacetanilic Acid.—Attempts to isolate sulfonylbisacetic anhydride after treatment of the acid with acetyl chloride, acetic anhydride, or phosphorus oxychloride under various conditions were not successful. However, preliminary work using only enough phosphorus oxychloride to form the half-chloride indicated that an anhydride might have been formed temporarily. The following procedure was devised to eliminate the necessity of isolating the anhydride.

Ten grams of sulfonylbisacetic acid and 2.5 cc. of phosphorus oxychloride were refluxed with 50 cc. of nitrobenzene in a round-bottom flask in an oil bath between 185–195° for one and one-half hours. After cooling the reaction mixture to 100°, 16 cc. of aniline was added resulting in the separation of a yellow crystalline mass. The filtered mass was treated with 200 cc. of hot water, filtered, and the filtrate treated with charcoal and refiltered. The filtrate after cooling yielded white plate-like crystals. Recrystallization of these from water resulted in a product melting slowly at 149° (dec.); yield 6.5 Gm. (34%). It had a neutral equivalent of 341 as compared to a calculated value of 350.

To 5 Gm. of the aniline salt was added 0.5 cc. of concentrated sulfuric acid in 30 cc. of water and the mixture heated until a clear solution resulted. The product was recrystallized once from alcohol resulting in fine needle-like crystals; m. p. 175–176° (dec.) (corrected); yield, 3.2 Gm. (87%).

Anal.—N. E. Calcd. for $C_{16}H_{11}O_5NS$: 257. Found, 254. Calcd.: N, 5.45; S, 11.39. Found: N, 5.35; S, 11.85.

N-Phenylsulfonylbisacetimide.—Three grams of sulfonylbisacetanilic acid was treated in 50 cc. of 1-nitropropane under anhydrous conditions with 0.7 cc. of phosphorus oxychloride by refluxing at 140–150° for one hour. Unreacted starting material separated on cooling, and was removed by filtration. The filtrate was concentrated in a vacuum at a low temperature. The resulting white, crystalline, somewhat gummy residue was washed with boiling water and recrystallized from acetone-alcohol solution two times. A crystalline compound melting at 243–245° (corrected) resulted; yield, 0.9 Gm. (32%). Titration of this compound with 0.1 *N* sodium hydroxide resulted in hydrolysis to the sodium salt of sulfonylbisacetanilic acid.

Anal.—Calcd. for $C_{16}H_9O_4NS$: N, 5.86; S, 13.39. Found: N, 5.81; S, 14.39.

7. Preparation of α,α′-Thiobispropionimide.—This product was prepared by heating 40 Gm. of the diammonium α,α′-thiobispropionate at 210–220° as previously described under the preparation of thiobisacetimide. The resulting oil was recrystallized three times from alcohol giving 13 Gm. of fairly large, shiny, crystalline plates which melted at 129° (corrected) (A).

Dilution of the alcoholic filtrate with water resulted in 13 Gm. of needle-like crystals which, after recrystallization from an alcohol-water mixture three times, melted at 69–72° (corrected) (B). Yields of both products were approximately equal with a total yield of 86% of the theoretical. Heating compound B in a test tube above an open flame resulted in compound A. Both compounds liberated ammonia when heated with a 10% solution of sodium hydroxide.

Anal.—Calcd. for $C_6H_9O_2NS$: N, 8.80; S, 20.12. Found for A: N, 8.76; S, 19.73; for B: N, 8.78; S, 20.37.

8. Preparation of α,α′-Thiobis-*n*-butyrimide.—Twenty-five grams of diammonium α,α′-thiobis-*n*-butyrate was heated as described under the preparation of thiobisacetimide. The resulting oil, after several recrystallizations from alcohol, yielded 8 Gm. (42%) of thin platelets; m. p. 116–117° (corrected). Only one crystalline compound was isolated even though possibility of *cis-trans* isomers exists.

Anal.—Calcd. for $C_8H_{13}O_2NS$: N, 7.49; S, 17.11. Found: N, 7.15; S, 17.52.

9. Synthesis of N-Phenyl-α,α′-thiobis-*n*-butyrimide: *Preparation of α,α′-Thiobis-n-butyric Anhydride.*—Twenty grams of α,α′-thiobis-*n*-butyric acid [*meso* form, m. p. 106–109° (3)] was refluxed for three hours with an excess acetyl chloride. After removal, under reduced pressure, of the excess acetyl chloride and formed acetic anhydride, the resulting oil was distilled under reduced pressure and the portion coming over between 149–151° at 15 mm. was collected; yield, 16 Gm. (85%). The oil solidified in an ice bath and melted at 18–19°. The neutral equivalent was 189.5 as compared to a calculated value of 188. The following physical constants were observed; $[\alpha]_{20}^{20}$ 1.1938, n_D^{20} 1.4942.

Similar results were obtained if the *racemic* form of the acid were used with the exception that the anhydride could not be solidified even at −10°.

α,α′-Thiobis-n-butanilic Acid.—Fifteen grams of the above anhydride was treated with 7.4 Gm. of aniline in 100 cc. of dry benzene. The crystals which separated on cooling were recrystallized several times from an alcohol-water mixture; m. p. 128–129° (corrected); yield, 15.5 Gm. (70%).

Anal.—N. E. Calcd. for $C_{14}H_{19}O_3NS$: 281. Found: 280.5. Calcd.: N, 4.98; S, 11.39. Found: N, 4.91; S, 11.85.

N-Phenyl-α,α′-thiobis-n-butyrimide.—Ten grams of the anilic acid obtained above was refluxed for three hours with an excess acetyl chloride. After the excess acetyl chloride and formed acetic anhydride were removed under reduced pressure, the oily residue was recrystallized from alcohol. Needle-like crystals were obtained which, after several recrystallizations, melted at 122–123° (corrected) (A).

When the alcohol filtrate was cooled in an ice-salt bath, additional crystals were obtained. These crystals were soluble in petroleum ether and alcohol while the others were not. Recrystallization of these from a benzene-petroleum ether mixture resulted in only a small portion of the higher melting compound, but concentration of the filtrate gave a compound melting at 61–62° (corrected) (B). The yield of each compound approximated 30% of the theoretical (2.5 Gm.).

Heating compound B in a test tube over an open flame resulted in compound A.

Anal.—Calcd. for $C_{14}H_{17}O_2NS$: N, 5.32; S, 12.17. Found for A: N, 5.40; S, 12.55; for B: N, 5.09; S, 13.30%.

10. Attempted Synthesis of α,α′-Sulfonylbis-*n*-butyrimide and N-Phenyl-α,α′-sulfonylbis-*n*-butyrimide via the Anhydride: *Preparation of α,α′-Sulfonylbis-n-butyric Anhydride.*—α,α′-Sulfonylbis-*n*-butyric acid was prepared by the method described for sulfonylbisacetic acid, with the exception that this acid would not form an insoluble barium salt. The acid was isolated by extraction with ether and purified by recrystallization from water. Yields approximated 55% of the theoretical.

Fifty-five grams of this acid [*meso* form, m. p. 156–158° (3)] was refluxed for eight hours with an excess acetyl chloride. Concentration of the reaction mixture under reduced pressure resulted in a crystalline product which was recrystallized two times from a benzene-petroleum ether mixture; m. p. 63–64° (corrected). Yield, 38 Gm. (76%). The compound gradually converted to the corresponding acid on standing.

Anal.—N. E. Calcd. for $C_8H_{12}O_4S$: 110. Found: 111.5.

α,α′-Sulfonylbis-n-butamic Acid.—A solution of 25 Gm. of the above anhydride in anhydrous benzene was saturated with dry ammonia gas. The resulting crude ammonium salt of the butamic acid was dissolved in an aqueous solution of sulfuric acid by boiling slightly. The resulting solution was cooled and the first needle-like crystals separating melted at 186–187° and were of unknown identity. Further concentration resulted in smaller crystalline needles which melted at 178–179° (dec.); yield, 12 Gm. (50%). These latter crystals were believed to be the desired amic acid. Neutral equivalent determinations gave varying results which averaged 10 above the calculated value of 237.

Anal.—Calcd. for $C_8H_{13}O_4NS$: N, 5.92; S, 13.50. Found: N, 6.09; S, 13.74.

Attempted Preparation of α,α′-Sulfonylbis-n-butyrimide.—All attempts to prepare the cyclic imide from the corresponding amic acid using acetyl chloride or phosphorus oxychloride were unsuccessful.

Attempted Preparation of N-Phenyl-α,α′-sulfonylbis-n-butyrimide.—Treating the anhydride obtained above with the calculated amount of aniline in a benzene solution resulted in a thick oil which could not be crystallized under various conditions, nor could the cyclic imide be isolated from a portion of the oil which had been refluxed with acetyl chloride.

11. Synthesis of α,α′-Thiobisisobutyrimide: *Preparation of Ethyl α-Bromoisobutyrate.*—Isobutyryl chloride was prepared by adding 132 Gm. of isobutyric acid to a very slight excess of thionyl chloride. The portion distilling at 92–94° was collected [reported b. p. 92° (8)]; yield, 119 Gm.

This product was brominated by adding a slight excess of bromine to the isobutyryl chloride and the mixture gradually heated to 100° over a period of six hours. The resulting mixture was saturated with CO_2 in order to remove the excess bromine and hydrogen bromide and then distilled under reduced pressure. The fraction coming over at 51–52° at 14 mm. pressure was collected (no record in the literature); yield, 186 Gm. Treating this product with absolute alcohol resulted in the desired ethyl ester. The fraction distilling at 59–60° at 14 mm. was collected [reported b. p. 70° at 20 mm. (9)]. The yield was 125 Gm. which approximated 43%, based on the isobutyric acid.

α,α′-Thiobisisobutyric Acid.—The procedure used was essentially that of Larsson (10). The diethylester of α,α′-thiobisisobutyric acid, which was an intermediate, was fractionated under reduced pressure. The fraction coming over between 135–138° at 8 mm. pressure was collected. The saponification equivalent for this fraction was found to be 132.4 as compared to the calculated value of 131.2 for the desired ester.

For the preparation of the acid, however, the unfractionated oil separated from the original reaction mixture was satisfactory. Saponification of this followed by acidification gave a product which, when recrystallized from water two times, melted over a range of 140–145° with an opalescent melt at 140–142° and a clear melt at 144–145°. Larsson lists the melting point at 142°. The neutral equivalent was 101.8 as compared to a calculated value of 103. The yield was 70% of the theoretical.

α,α′-Thiobisisobutyrimide.—Fifteen grams of the diammonium salt was heated in a flask between 230–250° for about one hour. The resulting oil was recrystallized several times from alcohol; m. p. 156–157° (corrected); yield, 7 Gm. (60%).

Anal.—Calcd. for $C_8H_{13}O_2NS$: N, 7.48; S, 17.11. Found: N, 7.34; S, 16.68.

12. Attempted Synthesis of α,α′-Thiobis(diethylacetic) Acid.—Following the same procedure used for the preparation of α,α′-thiobisisobutyric acid, all attempts have failed to yield the desired α,α′-thiobis(diethylacetic) acid from ethyl α-bromodiethylacetate.

13. Preparation of α,α′-Sulfonylbisisobutyrimide.—Two grams of α,α′-thiobisisobutyrimide was treated in acetic acid for three days at room temperature with 40% in excess of the calculated amount of 30% solution of hydrogen peroxide. Crystals appeared on the third day which were recrystallized two times from alcohol; m. p. 236–237° (uncorrected) (A); yield, 1.9 Gm. (81.5%).

This procedure, however, did not give the product consistently. Repeated attempts, even with ice cooling at the beginning of the reaction, resulted in a product melting at 222–223° with bubbling in the melting point tube (uncorrected) (B). Yields were about the same as for the higher melting compound.

Product B showed acidic properties and when neutralized with sodium hydroxide using phenolphthalein as an indicator and then acidified with hydrochloric acid, a product melting at 239–240° (uncorrected) resulted. A mixed melting point of this product with product A showed no depression in the melting point. There was approximately a 30% loss of material as a result of this recrystallization.

Further recrystallization of A from alcohol did not change its melting point, but if it was recrystallized by first neutralizing with sodium hydroxide followed by acidification with hydrochloric acid, the resulting crystals melted at 239–240° (uncorrected).

It was thought that perhaps compound B was α,α'-sulfonylbisisobutyramic acid, but the analysis did not confirm this assumption. The basis for the assumption was the loss of a gas, believed to be CO_2, at the melting point. The acid might readily lose CO_2 and convert to the cyclic imide.

Anal. for A.—Calcd. for $C_8H_{13}O_4NS$: N, 6.39; S, 14.61. Found: N, 6.40; S, 14.56.

Anal. for B.—Calcd. for $C_8H_{15}O_5NS$ (α,α'-sulfonylbisisobutyramic acid): N, 5.90. Found: N, 6.68.

14. Preparation of Sulfonylbisacetimide.—Following the procedure given in the preceding experiment (i.e., oxidation with 30% hydrogen peroxide at room temperature), slightly less than 1 Gm. of sulfonylbisacetimide was obtained from 4 Gm. of thiobisacetimide. This product melted at 206–207° (corrected). However, the product could not be obtained consistently by this procedure.

Anal.—Calcd. for $C_4H_5O_4NS$: N, 8.59; S, 19.63. Found: N, 8.57; S, 19.65.

15. Attempted Preparation of Other Sulfonyl Derivatives.—Attempts to oxidize α,α'-thiobispropionimide and α,α'-thiobis-*n*-butyrimide to the corresponding sulfones using a solution of hydrogen peroxide, as described above, failed to yield the desired products. Similar treatment of N-phenylthiobisacetimide resulted in sulfonylbisacetanilic acid.

16. Preparation of N-Methyl-α,α'-sulfonylbisisobutyrimide.—Three grams of α,α'-sulfonylbisisobutyrimide was treated in 30 cc. of water with 16 cc. of normal sodium hydroxide solution. To this mixture was added 3 cc. of dimethyl sulfate and the resulting mixture shaken vigorously for a short time. The mixture was then allowed to stand one hour with occasional shaking. The resulting mixture was then extracted with three 25-cc. portions of ether. The ether was evaporated and the residue recrystallized from water three to five times until a product melting at 115–116° (uncorrected) was obtained; yield, 2.1 Gm. (65%).

Anal.—Calcd. for $C_9H_{15}O_4NS$: N, 6.01. Found: N, 5.95.

17. Preparation of N-Methyl-α,α'-thiobisisobutyrimide.—Ten grams of α,α'-thiobisisobutyrimide was dissolved in 100 cc. of water and treated with 60 cc. of normal sodium hydroxide similar to the procedure above. The ether extraction yielded a liquid product with a characteristic camphoraceous odor. This product was distilled under reduced pressure and the portion coming over at 116–120° at 17 mm. pressure was collected. This portion still possessed the characteristic camphoraceous odor. The yield was 7.5 Gm. (70%).

Anal.—Calcd. for $C_9H_{15}O_2NS$: N, 6.96. Found: N, 6.76.

SUMMARY

1. The synthesis of 7 new alkyl- and aryl-substituted thiobisacetimides and 4 corresponding sulfonyl compounds are described, namely: (*a*) N-phenylthiobisacetimide, (*b*) N-benzoylthiobisacetimide, (*c*) sulfonylbisacetimide, (*d*) N-phenylsulfonylbisacetimide, (*e*) α,α'-thiobispropionimide, (*f*) α,α'-thiobis-*n*-butyrimide, (*g*) N-phenyl-α,α'-thiobis-*n*-butyrimide, (*h*) α,α'-thiobisisobutyrimide, (*i*) α,α'-sulfonylbisisobutyrimide, (*j*) N-methyl-α,α'-sulfonylbisisobutyrimide, and (*k*) N-methyl-α,α'-thiobisisobutyrimide.

2. Various intermediates are reported for the first time, namely: (*a*) sulfonylbisacetanilic acid and its aniline salt, (*b*) α,α'-thiobis-*n*-butyric anhydride, (*c*) α,α'-thiobis-*n*-butanilic acid, (*d*) α,α'-sulfonylbis-*n*-butyric anhydride, and (*e*) α,α'-sulfonylbis-*n*-butamic acid.

3. Preliminary pharmacological data indicated that most of the cyclic thio compounds were highly toxic and showed no anticonvulsant activity. N-methyl-α,α'-sulfonylbisisobutyrimide was relatively nontoxic but caused ataxia.

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Studies on Sex Variation to Picrotoxin in the Albino Rat*,†

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The subcutaneous LD_{50} of picrotoxin of the adult male albino rat was 87 per cent higher than for the female (5.48 versus 2.82 mg./Kg.); 458 rats were used in these determinations. No sex variation, however, was found in case of albino mice, guinea pigs, or Syrian hamsters. Castration of adult males or females lowered the resistance to picrotoxin. Daily administration of testosterone propionate over a period of ten days increased the resistance of normal and castrated young adult females. The typical sex difference persisted in normal rats in which both kidneys had been removed about eighteen hours prior to administration of picrotoxin.

THE FIRST mention of sex variation to picrotoxin was made by Holck (1), who reported an approximate median lethal dose of 5.9 mg. per Kg. for male and 3.5 for female hybrid rats. Although the matter of sex is not discussed, the data of Cole (2) also indicate a higher resistance of the albino male rat as compared with that of the female. A similarly marked sex difference in resistance to strychnine has been reported repeatedly (3, 4). However, in our preliminary studies, no sex variation was found in the case of another convulsant drug, namely, Metrazol.

EXPERIMENTAL

Fatal Dose Determinations in Albino Rats.—In the present study the median lethal doses (LD_{50}'s) for young adult albino rats of Wistar stock were established in the two sexes. The animals were not fasted and had been kept on a uniform diet in a temperature-controlled animal room. The picrotoxin was injected subcutaneously in the abdominal area, using a 0.2% solution in distilled water. Because it has been reported that the fatal dose of picrotoxin gradually decreased in mice as the room temperature is raised from 20° to 40° (5), we kept the temperature at 27–28° during this determination. After injection each animal was kept in an individual cage. The doses were increased in steps of 15% and a total of 179 males and 186 females were injected. The dosage range and observed mortalities are shown in Table I. To cover the whole range of fatalities the highest male dose of 7.31 mg./Kg. was added four weeks after concluding the rest of the one-day experiment carried out on both sexes simultaneously.

The LD_{50}'s, calculated by the double integration method proposed by Dragstedt and Lang (6), were 5.21 mg./Kg. for the males and 2.88 for the females, a ratio of 1.81 as compared with 1.69 for the smaller groups of hybrid rats already mentioned. The markedly higher male resistance may also be noted by comparing the fatalities with the doses of 3.66 and 4.20 mg./Kg.

Fatal Dose Determinations in Albino Mice.—In previous studies upon hexobarbital, although the rat exhibited a marked sex difference, no difference between the sexes was seen in mice (7). To ascertain if mice show any sex difference with picrotoxin, 8 groups of 11 male Swiss albino mice weighing 16½ to 24 Gm. each and 9 groups of 11 females ranging from 15 to 21 Gm. were injected with picrotoxin. The concentration was 0.1% in distilled water and the drug was injected subcutaneously in the area close to the base of the tail; the dosage increases were only 9.3%. The LD_{50} for the males was 4.92 mg./Kg. and for the females 5.01; the ratio between the sexes was only 0.98, indicating absence of sex difference to picrotoxin with this animal.

TABLE I.—PICROTOXIN MORTALITIES IN ALBINO RATS*

Picrotoxin, Mg./Kg.	Mortalities	
	Males	Females
2.10	...	1/31
2.41	...	6/31
2.77	...	16/31
3.18	...	19/31
3.66	0/31	26/31
4.20	2/31	31/31
4.82	13/31	...
5.54	19/31	...
6.37	25/31	...
7.31	24/24	...

* Nonfasted albino rats, 4–6 months old. Picrotoxin was administered subcutaneously from a 0.2% solution in distilled water.

The Age Factor in Sex Difference.—Before attempting any study on the influence of operations or medication on the sex difference to picrotoxin we first tried to ascertain the influence of age on each sex and on the sex difference by employing a method which necessitated fewer animals per test. In this type of test we injected a small dose of picrotoxin subcutaneously every ten minutes, using 10 different sites of injection. Absorption apparently was rapid for tremors generally were noted after 3 to 4 injections. In order to secure accurate measurements,

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we made the concentration of picrotoxin 0.075% in the case of 1- to 2-month-old rats, and 0.15 or 0.12% in the remainder, using distilled water as the solvent in all cases. The room temperatures were between 23° and 28°, but did not vary more than 2° for any single experiment. The data are shown in Table II and in Fig. 1.

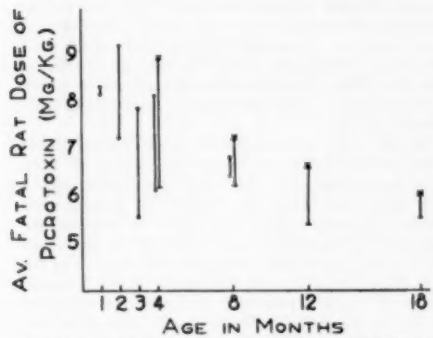

Fig. 1.—Plot of the average lethal doses of picrotoxin for data presented in Table II. The symbols • and × represent average lethal doses for male albino rats injected subcutaneously every ten minutes with 0.75 and 0.60 mg. of picrotoxin per Kg., respectively. The symbols O and - represent corresponding values for female rats. The extent of sex variation at each age is indicated by the length of the vertical line.

The importance of choosing the individual dose is brought out in these studies. If the dose is too small, some of the rats, especially the males, develop tolerance, and may require several times the usual number of doses, so that the standard error becomes very great. Such development of tolerance in some of the rats has previously been reported for pentobarbital and hexobarbital in the rat (8). If the dose is too great, the rat dies after a small number of doses; this is unsatisfactory from the statistical point of view; also, the sex difference may become obscured. The results also show that within each sex there seems to be a lowering of resistance as the rats advance in age from 2 to 8, or from 4 to 12 months. However, because the experiments were carried out at considerable intervals of time, additional tests would be required for confirmation. As to sex difference, this is absent at the age of 1 month, is noticeable at 2 months, and is most marked at the age of 3–4 months; although it seemed uncertain at the age of 8 months, it was apparently present at the age of 12 months, but not at 18. *In every case the tests upon the two sexes of the same age were carried out simultaneously.*

Fatal Dose Determinations in Syrian Hamsters and in Guinea Pigs.—Before attempting to use the multiple dose technique to study factors other than age and sex which might modify the response to picrotoxin in the rat, we applied it to a number of Syrian hamsters and of guinea pigs to ascertain if any sex variation existed in either animal species. The hamsters were 4 months old and were given 0.75 mg./Kg. (0.15% solution) subcutaneously every ten minutes. The average number of mg./Kg. required to produce death was 9.94 ± 0.73 for the 12 males and 10.69 ± 0.54 for the 12 females; the *t* factor is 0.83, hence no sex variation is present. Likewise, in young adult guinea pigs (410 to 502 Gm. males and 338 to 378 Gm. females) no sex difference could be detected. Owing to greater sensitivity to picrotoxin only 0.4 mg./Kg. was injected each time. The average for the 7 males was 4.36 ± 0.48 and for the 7 females 4.00 ± 0.09 mg.; the high standard error in case of the males is due to the fact that one male required 18 injections; the females were extremely uniform in their response. The *t* factor is only 0.7, indicating absence of sex difference.

Effects of Castration.—Four experiments were conducted to learn what effect, if any, castration would have upon the sex difference to picrotoxin in the rat. In the first group of tests, the rats were castrated at the age of two months. A single dose of picrotoxin was chosen which was supposed to kill a majority of the females and none or few of the males namely 3.66 mg./Kg., subcutaneously given when the rats were 4 months old. Of the normal females 8/16 and of the spayed ones 13/17 died; of the normal males, 0/16, and of the castrates, 3/15 succumbed.

TABLE II.—AGE FACTOR IN SEX DIFFERENCE TO PICROTOXIN IN ALBINO RATS

Age in Months	Mg./Kg. Every 10 Min.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error	*t* Factor
1	0.75	12	Males	8.31	0.74	
		12	Females	8.19	0.56	
2	...	12	Males	9.19	0.68	
		12	Females	7.25	0.56	2.11; $p = 0.04$
3	...	12	Males	7.88	0.45	
		12	Females	5.57	0.29	4.34
4	...	13	Males	8.14	0.39	
		14	Females	6.11	0.16	4.87
8	...	12	Males	6.81	0.26	
		12	Females	6.44	0.23	1.04
4	0.60	20	Males	8.94	0.77	
		20	Females	6.21	0.19	3.36
8	...	12	Males	7.25	0.48	
		12	Females	6.25	0.17	2.00; $p = 0.06$
12	...	12	Males	6.60	0.25	
		12	Females	5.40	0.20	3.83
18	...	10	Males	6.06	0.48	
		20	Females	5.55	0.14	1.09

Inasmuch as the evidence for decreased resistance by castration of each sex was inconclusive, the multiple injection technique was employed in three other sets of experiments. The rats were 4 months old and had been castrated when 2 months old. In the first phase of the study we administered 0.75 mg. of picrotoxin per Kg. each time, whereas in the second only 0.60 mg./Kg. was given. These tests were carried out four days apart and the results are summarized in Table III.

With the dose of 0.75 mg./Kg., castration of either sex apparently lowered the resistance to picrotoxin. With the dose of 0.60 mg./Kg. this effect is not seen so well in the males. One of the normal males developed tolerance and required 37 doses, hence the high standard error.

In our third group of experiments we employed 3.5- to 4.0-month-old rats which had been castrated at an earlier age, namely when they were 11 to 13 days old. The subcutaneous dose was 0.60 mg./Kg. every ten minutes when the rats were 3.7 to 4.7 months old. Tolerance developed in some of the 8 normal males, hence we discontinued injections into these when the average exceeded 10.86 mg./Kg. (18.1 injections). The results are presented in A of Table IV.

In a final group of tests we selected a higher dose, namely 0.671 mg./Kg., to avoid development of tolerance in the normal males. The rats were 5 to 6 months old and castration was done when they were 5 to 7 days old; only the castrated males are included because too few operated females survived to make their inclusion worth while. The results are given in B of Table IV.

The results in A indicate that castration of the males lowered their resistance to picrotoxin, but not to the level of that of the females (t = 4.13). However, according to B, not only was the resistance markedly lowered by castration of the males, but such castration brought the average below that of the normal females (p is less than 0.02). However, one cannot directly compare the results in Parts A and B because the ages, the time of castration, and the dosages all were different; also, the groups in A were rather small.

Effects of Administering Testosterone Propionate.—Having ascertained that male rats have a higher resistance to picrotoxin than females and that castration of the males lowers the resistance, we next attempted to learn to what extent, if any, administration of male sex hormone would influence the resistance of castrated males and females and of normal females. Increased resistance in such had previously been demonstrated by such medication to rats given hexobarbital and pentobarbital, in which case similar sex and castration of male effects were observed (9).

In our first experiment we administered single doses of picrotoxin to 4- to 4½-month-old rats. Two doses which normally would kill none or only a few males, but nearly all or all of the females were given subcutaneously. The dose of testosterone propionate (Perandren) was 0.298 mg. in sesame oil per rat and was given subcutaneously once a day for ten days preceding the picrotoxin administration and each day at a new site; an additional dose was given on the morning of picrotoxin administration. The fatalities are shown in Table V.

Thus, although the synthetic hormone definitely increased the resistance of the normal female rat to picrotoxin, it did not bring it up to that of the male.

In the second experiment we spayed a series of female rats at the age of 2 months and administered 0.298 mg. of testosterone propionate daily according to the above plan. When they were 6 months old

TABLE V.—EFFECT OF TESTOSTERONE PROPIONATE UPON THE RESISTANCE OF FEMALE ALBINO RATS TO PICROTOXIN

Dose, Mg./Kg.	Fatalities		
	Normal Males	Normal Females	Treated Females
3.66	0/15	14/15	2/15
4.20	2/15	15/15	7/15

TABLE III.—EFFECTS OF CASTRATION UPON THE NUMBER OF MILLIGRAMS OF PICROTOXIN NEEDED TO KILL ALBINO RATS

Mg./Kg.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error	t Factor
0.75	13	Normal males	8.14	0.39	
	20	Castrated males	6.94	0.32	2.47; $p = 0.04$
	14	Normal females	6.19	0.16	
	19	Spayed females	5.41	0.16	3.15
0.60	20	Normal males	8.94	0.77	
	20	Castrated males	7.50	0.26	1.76; $p = 0.09$
	20	Normal females	6.21	0.19	
	20	Spayed females	5.61	0.20	2.23; $p = 0.03$

TABLE IV.—EFFECT OF EARLY CASTRATION UPON THE RESISTANCE TO PICROTOXIN IN THE ALBINO RAT

	Mg./Kg. Every 10 Min.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error	t Factor
A.	0.60	8	Normal males	>10.86	...	...
		8	Males castrated when 11–13 days old	7.35	0.48	...
		7	Normal females	5.74	0.12	...
		7	Females spayed when 11–13 days old	5.40	0.19	...
B. (*a*)	0.671	13	Normal males	7.45	0.34	...
(*b*)	...	12	Males castrated when 5–7 days old	5.44	0.19	5.1 (*a* vs. *b*)
(*c*)	...	18	Normal females	6.11	0.19	2.5 (*b* vs. *c*)

the multiple injection method was used and each dose of picrotoxin was 0.60 mg./Kg. The results are shown in Table VI, A.

In the third experiment the technique was similar, but the age of the rats was 12½ to 13 months and the testosterone propionate dose was increased to 1.19 mg. per rat per injection to see if it was possible to influence the resistance in older males. The results are presented in Table VI, B. The number of injections of picrotoxin required to cause death is followed by the standard error.

The combined results indicate that whereas administration of male sex hormone markedly increased the resistance of spayed and to a lesser extent of normal female rats to picrotoxin, it had no effect upon that of normal males. The difference between normal males and treated females is not quite significant; $t = 1.63$, p about 0.1.

Effect of Sex Life Upon Resistance to Picrotoxin.—Because of reports by Agduhr (10), that sex life raises the resistance of rats to various drugs and poisons, Holck and Fink (11) previously studied this factor in case of pentobarbital. It was found that only when rats became pregnant did the ability to build up tolerance distinctly increase, this increase persisting through the lactating period. With picrotoxin the following tests were carried out upon pregnant rats. A single dose was chosen which was expected to kill all or nearly all normal female rats, but only a few of the males; the medication was by subcutaneous injection and the concentration 0.2% in distilled water. The pregnancies varied from early to late in each group. The results are presented in Table VII.

TABLE VII.—EFFECT OF PREGNANCY UPON MORTALITY WITH PICROTOXIN IN THE ALBINO RAT

Dose, Mg./Kg.	Females		Males, Unmated
	Unmated	Pregnant	
4.82	7/9	9/9	3/9
5.54	9/9	9/9	3/9

Thus, pregnancy did not have any appreciable influence upon the resistance to picrotoxin.

In a second experiment the resistance of mated and unmated males, and of lactating mothers and unmated females were compared. Each male was placed for five weeks in a cage which to begin with contained 3 female rats; as soon as a female appeared to be pregnant it was removed. No male at the time of picrotoxin administration was with less than one female. The baby rats of the lactating mothers were from 4 to 18 days old. We employed the multiple injection method and administered 0.75 mg./Kg. per injection every ten minutes under the skin; at the time of picrotoxin medication the animals were 6 months old. The average number of mg./Kg. with their standard errors are shown in Table VIII.

TABLE VIII.—EFFECTS OF MATING MALE RATS AND OF LACTATING UPON RESISTANCE TO PICROTOXIN IN THE ALBINO RAT

Mg./Kg. Every 10 Min.	No. of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error
0.75	18	Unmated males	7.88	0.29
	18	Mated males	8.02	0.29
	17	Unmated females	6.98	0.20
	17	Lactating females	7.46	0.25

Thus, neither mating of the males nor lactating influenced the resistance to picrotoxin; the t factor for the two female groups is only 1.52, hence p exceeded 0.1. A t factor of 2.55 (p less than 0.02) shows the typical sex variation between unmated males and females.

Effect of Blocking the Reticulo-Endothelial System with Thorotrast.—Inasmuch as the reticulo-endothelial system has been shown to be an important factor in sulfonamide conjugation by Maher (12), an attempt was made to ascertain if this system might be involved in the sex variation to picrotoxin. Following the plan of Maher, intravenous injections of the blocking agent, thorotrast, were made; this agent is a stabilized colloidal suspension containing 24% to 26% of thorium dioxide. Having established that 6 cc./Kg. was fatal to a large proportion of the rats, we injected only 3 cc./Kg. into a femoral vein under ether anesthesia on the afternoon prior to administering picrotoxin; however, even with this dose 2 of the females died overnight. The multiple injection technique was employed and a dose of 0.60 mg. of picrotoxin per Kg. was adopted; the rats were four to five months old. The average number of mg./Kg. with the standard error are presented in Table IX, A.

In a repetition experiment we injected the thorotrast more slowly and gave picrotoxin on the third day following these injections. This gave the animals more time to recover from the effects of the ether anesthesia and the incision. Inasmuch as the thorotrast effects tend to wear off—though the substance is still in the body—it was considered unwise to use a longer interval of time between thorotrast medication and picrotoxin injections. None of the animals died. The individual dose this time was 0.671 mg. of picrotoxin per Kg. (0.134% solution) and the rats were 4.0 to 4.7 months old. The results are recorded in Table IX, B.

The t factor in A was 2.18, $p = 0.04$, indicating that shortly after thorotrast medication sex difference was not completely abolished. B shows that when full thorotrast blocking had occurred, the sex variation to picrotoxin completely disappeared.

TABLE VI.—EFFECTS OF TESTOSTERONE PROPIONATE UPON THE RESISTANCE OF NORMAL MALE AND FEMALE RATS AND OF SPAYED RATS TO PICROTOXIN

	Mg./Kg. Every 10 Min.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error	t Factor
A	0.60	9	Spayed females	5.40	0.17	
		9	Hormoned treated females	7.56	0.25	7.1
B		13	Normal males	7.02	0.29	
		13	Hormone treated males	6.96	0.19	
		14	Normal females	5.70	0.15	
		14	Hormone treated females	6.36	0.26	2.3; $p = 0.03$

TABLE IX.—EFFECTS OF BLOCKING THE RETICULO-ENDOTHELIAL SYSTEM WITH THOROTRAST UPON THE SEX VARIATION TO PICROTOXIN IN THE ALBINO RAT

	Mg./Kg. Every 10 Min.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error
A.	0.60 (after 18 hrs.)	12	Treated males	6.11	0.49
		12	Treated females	4.90	0.18
B.	0.671 (after 64 hrs.)	15	Treated males	7.72	0.28
		15	Treated females	8.05	0.36

Effects of Keeping Picrotoxin in Blood Serum.—To ascertain if the blood contained substances which would detoxify picrotoxin we centrifugalized blood serum from male rats. Picrotoxin was dissolved in this serum and kept for about thirty minutes prior to injection. Similarly, another sample was dissolved in distilled water and kept there for thirty minutes. The concentrations of picrotoxin were 0.1%. A single dose of 3.75 mg. of picrotoxin per Kg. was administered subcutaneously to each rat. The mortalities which resulted with the 7- to 7½-month-old female rats are presented in Table X.

Thus, not only did fewer rats die, but those that did survived longer when picrotoxin had been stored in blood serum. Hence, apparently, the picrotoxin was altered by standing in blood serum.

Effect of Double Nephrectomy on Sex Variation to Picrotoxin in the Albino Rat.—To ascertain if the kidney played any important role in the sex variation to picrotoxin, sixteen 4- to 5-month-old rats of each sex were double nephrectomized under ether anesthesia the afternoon prior to giving 0.60 mg. of picrotoxin per Kg. subcutaneously every ten minutes until each rat had died. The average number of mg./Kg. with the males was 6.07 ± 0.31 and with the females 4.54 ± 0.14. The t factor of 4.39 indicates that the sex variation in double nephrectomized rats is of the same order as in normal animals.

Effects of Epinephrine and of Insulin Medication on Resistance to Picrotoxin and to Hexobarbital.—In view of the report by Reinhard (13), that preliminary medication with epinephrine lowered the resistance of mice to hexobarbital, and because the adrenals are much larger in female (see subsequent data) than in male rats, an experiment was carried out to see whether or not such preliminary epinephrine medication would alter the male rat response to picrotoxin. Twelve rats, 4 to 6 months of age, were given 0.05 mg. of epinephrine hydrochloride per Kg., intramuscularly, ten minutes prior to administering 0.75 mg./Kg. of picrotoxin, subcutaneously, every ten minutes. The epinephrine solution was 0.02%. Twelve control rats were given a similar volume of 0.9% sodium chloride solution prior to picrotoxin. That the epinephrine dose was safe is indicated by the fact that one rat, which was given 1 mg./Kg., survived. With two, however, death ensued in seven minutes. The results are presented in Table XI, A.

TABLE X.—EFFECT ON MORTALITIES IN ALBINO FEMALE RATS OF STORING PICROTOXIN IN BLOOD SERUM FOR 30 MINUTES PRIOR TO ADMINISTRATION

Drug stored in water	9/9	Average death time: 39 minutes
Drug stored in blood serum	4/10	Average death time: 58 minutes

Insulin premedication likewise was shown by Reinhard (13) to prolong the hypnosis with hexobarbital in mice. Therefore, preliminary administration of insulin to male rats was tried to study its effect on resistance to picrotoxin. Ten 4- to 4½-month-old rats were given 2 units of insulin per Kg., intramuscularly, about an hour and a half after separating the nonfasted rats and exactly twenty minutes prior to beginning the picrotoxin injections. Owing to the higher resistance of the rat than the mouse to insulin, we chose this dose, which is twice the mouse dose given by Reinhard (13) and by Holck (14). The latter found that neither epinephrine nor insulin influenced the fatal dose of hexobarbital in mice. The multiple dose method was adopted and the picrotoxin dose was again 0.75 mg./Kg. The results are presented in Table XI, B.

These results clearly show that neither epinephrine nor insulin as here administered exerted any influence upon the resistance to picrotoxin.

In view of these negative results it seemed desirable to ascertain if rats behaved like mice to hexobarbital when pretreated with insulin, as reported for mice by Reinhard. We therefore administered 5 units of insulin per Kg. intramuscularly to 12 nonfasted male rats and to 11 nonfasted females, using a solution containing 5 units of insulin per cc. Twenty minutes later 100 mg. of sodium hexobarbital per Kg. was administered intraperitoneally (5 cc. of 2% solution in distilled water). The controls were given preliminary 0.9% sodium chloride solution. The rats were 4 to 4½ months old. The average number of minutes of loss of the righting reflex in the four groups, with the standard error, is presented in Table XII.

These results indicate that insulin did not significantly influence either the male or the female resistance to hexobarbital; the t factor in case of the females is 1.77, or $p = 0.09$. The usual sex variation is present in both the untreated and the treated rats. Apparently, rats pretreated with insulin differ from similarly treated mice in their resistance to hexobarbital.

TABLE XI.—EFFECTS OF PRELIMINARY EPINEPHRINE HYDROCHLORIDE OR OF INSULIN MEDICATION UPON THE RESISTANCE TO PICROTOXIN IN THE ALBINO RAT

	Mg./Kg. Every 10 Min.	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error
A	0.75	12	Control males	6.56	0.20
		12	Epinephrinized males	6.69	0.38
B	0.75	10	Control males	6.00	0.20
		10	Insulinized males	6.00	0.27

TABLE XII.—EFFECT OF PREMEDICATION WITH INSULIN UPON THE TIME OF LOSS OF RIGHTING REFLEX WITH HEXOBARBITAL IN THE NORMAL ALBINO RAT[a]

Number of Rats	Sex	Average Loss of Righting Reflex, in Minutes	Standard Error
12	Untreated males	42.3	2.9
12	Insulinized males	45.2	2.1
11	Untreated females	92.4	5.1
11	Insulinized females	107.2	6.6

[a] 100 mg. of sodium hexobarbital per Kg. was injected intraperitoneally twenty minutes after administering 5 units of insulin per Kg. intramuscularly.

Effects of Adrenal Cortex Hormone Medication upon Resistance to Picrotoxin, upon Size of the Adrenal Gland, and upon Storage of Ascorbic Acid in This Gland.—Finally, because female rats possess much larger adrenal glands than males, we studied the possible influence of administering adrenal cortex hormone. For fifteen days prior to, and on the morning of, picrotoxin administration, we injected 0.2 mg. of corticosterone acetate (Percorten) in sesame oil per rat daily by muscle. The controls were given sesame oil only. The rats were $4^1/_2$ months old at the beginning of the hormone medication. Injections of 0.70 mg. of picrotoxin per Kg. (0.14% solution) were given under the skin every ten minutes until death. Total ascorbic acid determinations were made after death by the method of Musulin and King (15), as modified by Morell (16). The average number of mg./Kg. needed to cause death, with the standard error, and the relative adrenal and ascorbic acid weights are tabulated in Table XIII.

In the last group one rat developed tolerance and was in fair condition after receiving 19.6 mg./Kg. (28 injections) when injections were discontinued. The figures in the last two columns represent all 15 rats. The results indicate that in our stock the female adrenal glands are larger in proportion to those of the males than reported by Donaldson (17); the value for the control males agrees closely with that previously reported by Holck, *et al.* (7), namely, 114 mg./Kg. of body weight. Administration of corticosterone altered the resistance to picrotoxin in neither sex. However, in the males it increased not only the adrenal weight proportion, but also the ascorbic content per unit of adrenal weight to a value close to that seen in the females. It is not possible to conclude that medication with the adrenal cortex hormone abolished or diminished the sex variation to picrotoxin because the determinations upon the females were carried out six days after those upon the males and not simultaneously; our own experience with picrotoxin and recent comprehensive work indicates that a difference of a few days may make a considerable difference in the reponse of rats to various drugs (18).

DISCUSSION

Higher male rat resistance to picrotoxin is seen either by determining the median lethal doses for the two sexes or by comparing the average number of injections needed to produce death when a small dose is injected subcutaneously every ten minutes. Although the latter method is more expedient, it is not ideal because even when the dosage supposedly is well chosen, some rats may develop tolerance and require several times the average dose for the remainder of the group; thus, if included, the standard error becomes very great. A method by which in part to overcome this difficulty is the cumbersome one tried with pentobarbital (but not in the present experiments), in which case one starts with a small dose and increases it by a small percentage each time. Likewise, it is not practical to employ very large numbers of each sex to determine what proportion develop tolerance on any given dose.

The appearance of sex difference in resistance to picrotoxin as maturity approaches and its presence for at least the first year of the rat's life is in agreement with other studies upon sex variation in the rat, as, for example, with pentobarbital or hexobarbital (7, 8), or the studies upon strychnine by Poe, *et al.* (3), in which latter the sex variation was indicated at the age of 6 weeks, but much more marked when the rats were 18 weeks old. That the rat again is unique in exhibiting such sex difference is shown by the fact that it was absent in mice, hamsters, and guinea pigs, just as it was absent in mice, guinea pigs, rabbits, cats, and dogs with hexobarbital (7).

Castration of either sex lowered the resistance to picrotoxin. Studies upon the males indicate that the difference becomes greater when castration is made at the age of one week rather than at two months; resistance to picrotoxin of adult male rats castrated at one week of age was even lower than that of the normal females. The effect of castration of males upon resistance to picrotoxin follows that seen with the similar higher male resistance to short acting barbiturates, such as hexobarbital or pentobarbital (7, 8). However, in the case of pentobarbital spaying of the females, it did not lower, but increased the resistance in contrast to what is here seen with picrotoxin.

Pretreatment with daily doses of testosterone propionate raised the resistance to picrotoxin in

TABLE XIII.—EFFECTS OF CORTICOSTERONE ACETATE PREMEDICATION UPON RESISTANCE TO PICROTOXIN, UPON ADRENAL GLAND SIZE, AND UPON STORAGE OF ASCORBIC ACID IN THE ADRENAL GLAND OF THE ALBINO RAT

Mg./Kg. Every 10 Min	Number of Rats	Sex	Fatal Dose, Mg./Kg.	Standard Error	Adrenals Mg./Kg.	Ascorbic Acid as % of Adrenals
0.70	15	Control males	7.61	0.29	108	0.083
	15	Hormone treated males	7.33	0.34	154	0.123
	15	Control females	7.19	0.27	324	0.114
	15	Hormone treated females	7.00	(14 rats only)	353	0.110

normal and especially in spayed females; generally, however, it did not bring the resistance of the normal females quite up to that of the normal males. Such effect of the synthetic male hormone is similar to the effects produced in normal and spayed females upon their response to those barbiturates to which male rats have higher resistance (9).

Although with pentobarbital, pregnant and lactating females in previous experiments were more resistant, with picrotoxin such rats had the same resistance as unmated females. Also, mating for five weeks had no influence upon male rat resistance, agreeing with our previous findings with pentobarbital and longer periods of mating (11).

The disappearance of sex variation to picrotoxin when the reticulo-endothelial system was completely blocked by thorium dioxide and the loss of potency of picrotoxin which had been stored for half an hour in male rat blood serum seem promising leads for further investigation. The kidneys seemingly played no part in the sex variation, just as they did not with hexobarbital in earlier studies (7). Likewise, premedication with epinephrine or insulin, previously reported to make mice more sensitive to hexobarbital (13), failed to influence resistance to picrotoxin; that also holds true for 15-day premedication with corticosterone.

SUMMARY

1. One hundred and seventy-nine adult male and 186 female rats were divided into groups and injected subcutaneously with graded doses of picrotoxin. The median lethal dose (LD_{50}) for the males was 5.21 and the females 2.88 mg./Kg., giving a ratio of 1.81.

2. Sex variation to picrotoxin was absent in one-month-old rats, indicated at the age of 2 months and most marked in 3- to 6-month-old rats; it was still demonstrable at the age of one year. In these determinations a small dose of picrotoxin was injected subcutaneously every ten minutes until each rat had died and the average number of doses or injections with its standard error calculated.

3. Results by this method showed that castration of either sex lowered the resistance to picrotoxin; in one series of males this was shown to be most marked when the rats were castrated when they were only 5 to 7 days old.

4. Administration of testosterone propionate in sesame oil subcutaneously for ten to fifteen days increased the resistance to picrotoxin of normal and, especially, of spayed female rats, but did not influence the effect of picrotoxin upon one-year males.

5. During the period of pregnancy and lactation the resistance to picrotoxin was unaltered. Mating of male rats for five weeks likewise failed to influence the resistance.

6. Blocking the reticulo-endothelial system with thorium dioxide completely abolished the sex variation to picrotoxin.

7. Storing picrotoxin for half an hour in male rat blood serum lowered the potency of the drug.

8. Double nephrectomy did not alter the sex difference.

9. Preliminary medication with epinephrine did not change the resistance to picrotoxin. Preliminary treatment with insulin failed to alter the effect of either picrotoxin or hexobarbital.

10. Daily medication for fifteen days with corticosterone acetate did not influence the resistance of either sex to picrotoxin. In the males it increased the relative weight of the adrenals and the percentage of ascorbic acid stored in these glands.

11. No sex variation to picrotoxin could be detected in albino mice, guinea pigs, or Syrian hamsters.

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The Effect of Fineness of Sulfadiazine and Wetting Agents on Diffusion from Ointment Bases*

By BENJAMIN LEVY† and C. LEE HUYCK‡

The diffusion rates of microcrystalline and powdered sulfadiazine from several different types of ointment bases have been compared. From hydrophilic ointment bases microcrystalline sulfadiazine diffused more rapidly than the powdered drug. The addition of sodium lauryl sulfate to white ointment containing sulfadiazine increased slightly the liberation of microcrystalline sulfadiazine.

SINCE the advent of sulfonamide therapy, many reports have appeared on the relative clinical value of sulfonamides in various ointment bases with and without wetting agents. Little has been published relative to the effect of fineness of powder on the diffusion of sulfonamides from various ointment bases. The results of some comparative *in vitro* diffusion tests on the effect of the fineness of powder on the diffusion of sulfadiazine from various ointment bases, with and without wetting agent by the method of Waud and Ramsay (1), are reported here.

HISTORY

Huyck, Hirose, and Reyes (2), in a comprehensive review of the diffusion of sulfonamides from emulsified ointment bases, reported:

(*a*) Twenty-four of the investigators mentioned in the review compared the results of clinical investigations of sulfonamides suspended in water-in-oil, grease, and water-soluble bases. Sixty-two and one-half per cent preferred the oil-in-water emulsion base for general use except for burns. Eight and thirty-five hundredths per cent preferred the water-in-oil base, 4.16% preferred the grease base, 16.7% preferred the water-miscible base, while 8.35% maintained that the oil-in-water emulsion base gave as good results as the water-in-oil emulsion base.

(*b*) *In vitro* tests indicated sulfa drugs suspended in an oil-in-water emulsion-type ointment base diffused more readily than sulfa drugs suspended in the water-in-oil emulsion-type ointment base.

(*c*) *In vitro* tests indicated that diffusion increased with the solubility of the base.

(*d*) The addition of a large amount of the wetting agent Aerosol O. T. increased the diffusion of sulfa drugs suspended in the water-in-oil emulsion-type base.

(*e*) The addition of lanolin to triethanolamine emulsions containing sulfathiazole increased slightly the diffusion of sulfathiazole.

McKee, Sulzberger, Herman, and Baer (3) found that the best penetration of tracer substances through the skin was effected by means of vehicles which utilized the combined and reciprocal action of water, propylene glycol, and interfacial tension-reducing agents. Better penetration was effected by aqueous solutions, particularly when interfacial tension-reducing agents were added.

Alvaro (4) found the local application of powdered and microcrystalline sulfonamides in hydrophilic ointment bases containing wetting agents produced satisfactory penetration of the cornea.

Franceschetti, Blum, and Valerio (5) found that the addition of sodium lauryl sulfate to a solution or pomade containing dimethyl acroylsulfonamide produced a high ocular concentration of the sulfanilamide when administered either by iontophoresis or in the form of a pomade. It is only by combining the sulfanilamide with a wetting agent that a sufficient concentration of the sulfonamide can be obtained to be of therapeutic value for the eye.

Sarria (6) found that in ointments containing hydrocarbon bases, sodium lauryl sulfate has to be used in a concentration of 3%, at which concentration it proved irritating. In greaseless creams the concentration of sodium lauryl sulfate can be reduced to a very low percentage—less than 1/4%—as a high concentration is not necessary for successful results. A higher concentration of sodium lauryl sulfate might prove useful and desirable to dermatologists in their fight to destroy bacteria, since the lowered surface tension produced (*a*) larger areas of contact with bactericidal agents, (*b*) higher detergency of diseased areas, and (*c*) compatibility with serum discharges and simple removal by water.

EXPERIMENTAL

The method of Waud and Ramsay (1) was used to evaluate the diffusion of ordinary and microcrystalline sulfadiazine[1] from hydrophilic and nonhydrophilic ointment bases with and without the wetting agent, sodium lauryl sulfate.

Ringer's solution containing 4% agar was prepared. To this was added 5% Ehrlich's solution, which consists of 2 Gm. paradimethylaminobenzaldehyde, 50 cc. of concentrated hydrochloric acid, and a sufficient quantity of distilled water to make 1000 cc. This solution when in contact with sulfadiazine turns yellow. After being placed in test tubes and allowed to gel, a layer of the vehicle containing the sulfadiazine was placed over the gel and the extent of diffusion measured at definite intervals.

The two ointment bases, used for the ordinary and microcrystalline sulfadiazine, were representative ointment bases of the United States Pharmacopœia. They are the white ointment base and the hydrophilic ointment base of United States Pharmacopœia XIII, Decennial Revision.

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† Submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Pharmacy, Columbia University.

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[1] The ordinary and the microcrystalline sulfadiazines were furnished by Calco Chemical Company, Bound Brook, N. J., through the courtesy of Dr. Noel E. Foss.

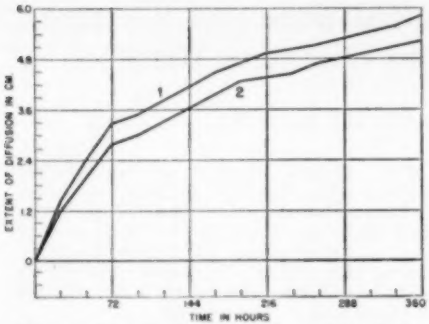

Fig. 1.—Five % microcrystalline sulfadiazine in hydrophilic ointment base versus 5 % sulfadiazine in hydrophilic ointment base, at room temperature: The hydrophilic ointment containing the microcrystalline sulfadiazine No. 1 has diffused more than ordinary sulfadiazine in hydrophilic ointment base No. 2 at room temperature.

The formulas of the ointments are as follows:

FORMULA 1

5% Microcrystalline sulfadiazine in hydrophilic ointment

Microcrystalline sulfadiazine	5.00 Gm.
Stearyl alcohol	23.75 Gm.
White petrolatum	23.75 Gm.
Glycerin	11.40 Gm.
Sodium lauryl sulfate	0.95 Gm.
Methyl para hydroxybenzoate	0.025 Gm.
Propyl para hydroxybenzoate	0.014 Gm.
Distilled water	35.150 Gm.
	100.039 Gm.

FORMULA 2

5% Sulfadiazine in Hydrophilic Ointment, U. S. P. XIII

Sulfadiazine	5.00 Gm.
Stearyl alcohol	23.75 Gm.
White petrolatum	23.75 Gm.
Glycerin	11.40 Gm.
Sodium lauryl sulfate	0.95 Gm.
Methyl para hydroxybenzoate	0.025 Gm.

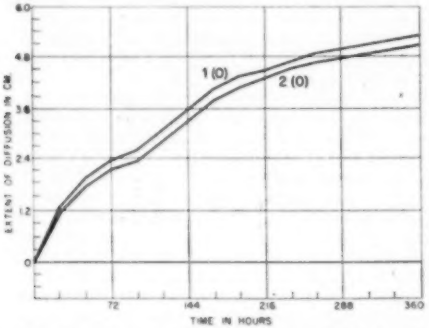

Fig. 2.—Five % microcrystalline sulfadiazine in hydrophilic ointment base versus 5 % sulfadiazine in hydrophilic ointment base after one month at 37.5°: The hydrophilic ointment containing microcrystalline sulfadiazine 1 (0) diffused greater than ordinary sulfadiazine in hydrophilic ointment base 2 (0) at 37.5°.

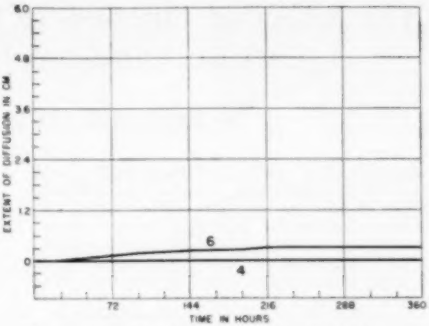

Fig. 3.—Five % microcrystalline sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base versus 5 % sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base, both ointments at room temperature: The ointment containing microcrystalline sulfadiazine and 1 % sodium lauryl sulfate in white ointment base (6) diffused more than the 5 % ordinary sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base (4) at room temperature.

Propyl para hydroxybenzoate	0.014 Gm.
Distilled water	35.150 Gm.
	100.039 Gm.

FORMULA 3

5% Sulfadiazine in White Ointment, U. S. P.

Sulfadiazine	5.00 Gm.
White petrolatum	85.50 Gm.
Wool fat	4.75 Gm.
White wax	4.75 Gm.
	100.00 Gm.

FORMULA 4

5% Sulfadiazine, 1% Sodium Lauryl Sulfate in White Ointment, U. S. P.

Sulfadiazine	5.00 Gm.
Sodium lauryl sulfate	1.00 Gm.
White petrolatum	84.60 Gm.
Wool fat	4.70 Gm.
White wax	4.70 Gm.
	100.00 Gm.

FORMULA 5

5% Microcrystalline Sulfadiazine in White Ointment U. S. P.

Microcrystalline sulfadiazine	5.00 Gm.
White petrolatum	85.50 Gm.
Wool fat	4.75 Gm.
White wax	4.75 Gm.
	100.00 Gm.

FORMULA 6

5% Microcrystalline Sulfadiazine, 1% Sodium Lauryl Sulfate in White Ointment U. S. P.

Microcrystalline sulfadiazine	5.00 Gm.
Sodium lauryl sulfate	1.00 Gm.
White petrolatum	84.60 Gm.
Wool fat	4.70 Gm.
White wax	4.70 Gm.
	100.00 Gm.

The diffusion of sulfadiazine from the sulfadiazine ointments was measured and the results are recorded. Ointments of opposite and of similar types of sulfadiazine are considered in the same figure for the purpose of comparison.

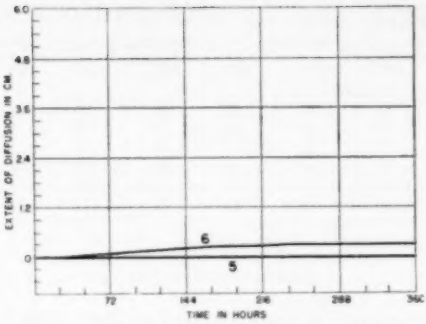

Fig. 4.—Five % microcrystalline sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base versus 5 % microcrystalline sulfadiazine in white ointment base, both ointments at room temperature: The ointment containing microcrystalline sulfadiazine and 1 % sodium lauryl sulfate in white ointment base (6) diffused more than microcrystalline sulfadiazine in white ointment base (5) at room temperature.

Since an ointment stored for one month at 37.5° represents an ointment stored for a longer period of time at room temperature, both ointments were examined for diffusion.

It was found that the official hydrophilic ointment base separated after one month at 37.5°, but readily returned to its normal consistency on cooling to room temperature and mixing.

DISCUSSION

On the basis of *in vitro* diffusion experiments, Waud and Ramsay (1) and many other workers have suggested that the practice of incorporating water-soluble drugs in fatty bases or water-in-oil emulsions should be discontinued. Results reported here are in general agreement with this. However, sulfadiazine with wetting agent in white ointment base is of some value. Huyck, Hirose, and Reyes (2) found that the addition of the wetting agent Aerosol O. T.

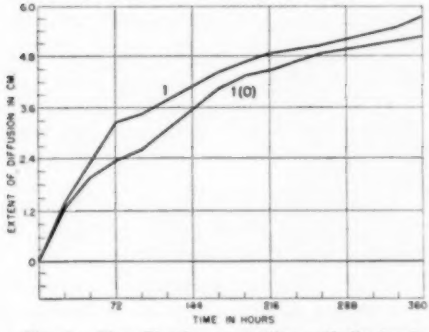

Fig. 5.—Five % microcrystalline sulfadiazine in hydrophilic ointment base at room temperature versus 5 % microcrystalline sulfadiazine in hydrophilic ointment base kept at 37.5° for one month: The ointment of microcrystalline sulfadiazine in a hydrophilic base (1) at room temperature diffused more than the ointment containing the same ingredients 1 (0) kept at 37.5° for one month.

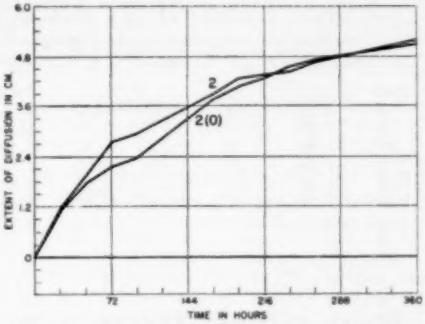

Fig. 6.—Five % sulfadiazine in hydrophilic ointment base at room temperature versus 5 % sulfadiazine in hydrophilic ointment base kept at 37.5° for one month: The ointment of ordinary sulfadiazine in a hydrophilic base (2) at room temperature gave a slightly greater diffusion than the ointment 2 (0) containing the same ingredients at 37.5° for one month.

increased the diffusion of sulfa drugs suspended in the water-in-oil emulsion-type base.

Sarria (6) found that in greaseless creams the concentration of sodium lauryl sulfate can be reduced to less than ½% to get successful results. McKee, Sulzberger, Herman, and Baer (3) found better penetration was effected by aqueous solutions, particularly when interfacial tension reducing agents were added. Franceschetti, Blum, and Valerio (5) found that it is only by combining the sulfanilamide with a wetting agent that it is possible, by simple application of a pomade, to obtain a sufficient concentration of active substance in the eye for efficacious therapeutic action. Alvaro (4) found that powdered and microcrystalline sulfonamides with substances which break the surface tension produce a greater penetration of sulfonamides through the

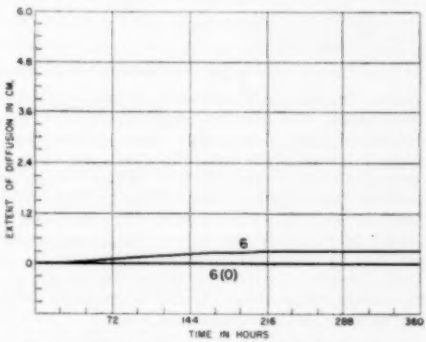

Fig. 7.—Five % microcrystalline sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base at room temperature versus 5 % microcrystalline sulfadiazine containing 1 % sodium lauryl sulfate in white ointment base at 37.5° for one month: The microcrystalline containing 1% sodium lauryl sulfate in white ointment base (6) at room temperature has a slightly greater diffusion than the one 6 (0) containing the same ingredients at 37.5°.

TABLE I.—EXTENT OF DIFFUSION IN CENTIMETERS OF SULFADIAZINE OINTMENTS KEPT AT ROOM TEMPERATURE

	Time in Hours										
	24	48	72	96	168	192	216	240	264	336	360
Sample	Diffusion in Centimeters										
A	1.40	2.40	3.30	3.50	4.50	4.80	5.00	5.10	5.20	5.60	5.90
1 B	1.40	2.40	3.20	3.40	4.40	4.80	4.80	4.90	5.00	5.50	5.70
Av.	1.40	2.40	3.25	3.45	4.45	4.80	4.90	5.00	5.10	5.55	5.80
A	1.20	2.00	2.80	3.00	4.00	4.30	4.40	4.45	4.70	5.10	5.25
2 B	1.15	1.95	2.70	2.90	3.80	4.20	4.30	4.40	4.65	5.00	5 15
Av.	1.17	1.97	2.75	2.95	3.90	4.25	4.35	4.42	4.67	5.05	5.20
A	0	0	0	0	0	0	0	0	0	0	0
3 B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0
4 B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0
5 B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0.10	0.15	0.20	0.30	0.30	0.30	0.30	0.30	0.30	0.30
6 B	0	0.00	0.05	0.10	0.20	0.20	0.25	0.30	0.30	0.30	0.30
Av.	0	0.05	0.10	0.15	0.25	0.25	0.28	0.30	0.30	0.30	0.30

TABLE II.—EXTENT OF DIFFUSION IN CENTIMETERS OF SULFADIAZINE OINTMENTS KEPT AT 37.5° FOR ONE MONTH

	Time in Hours										
	24	48	72	96	168	192	216	240	264	336	360
Sample	Diffusion in Centimeters										
A	1.30	2.00	2.40	2.70	4.10	4.40	4.60	4.80	5.00	5.30	5.40
1 (0) B	1.30	1.90	2.30	2.50	4.00	4.30	4.40	4.60	4.80	5.10	5.20
Av.	1.30	1.95	2.35	2.60	4.05	4.35	4.50	4.70	4.90	5.20	5.30
A	1.20	1.80	2.20	2.40	3.90	4.20	4.40	4.60	4.80	5.10	5.20
2 (0) B	1.10	1.70	2.10	2.30	3.70	4.00	4.20	4.50	4.60	4.90	5.00
Av.	1.15	1.75	2.15	2.35	3.80	4.10	4.30	4.55	4.70	5.00	5.10
A	0	0	0	0	0	0	0	0	0	0	0
3 (0) B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0
4 (0) B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0
5 (0) B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0
6 (0) B	0	0	0	0	0	0	0	0	0	0	0
Av.	0	0	0	0	0	0	0	0	0	0	0

cornea than without the surface tension lowering substances. The results reported here show that microcrystalline sulfadiazine with sodium lauryl sulfate in hydrophilic base diffused readily. Microcrystalline sulfadiazine in white ointment base with sodium lauryl sulfate diffused slightly right after preparation, but did not diffuse after one month at 37.5°. A possible explanation is that small particles coalesce to form large particles on storage. Good clinical results were obtained in treating infections of the cornea when microcrystalline sulfonamides were incorporated in hydrophilic ointments.

SUMMARY AND CONCLUSIONS

1. Microcrystalline sulfadiazine was liberated faster and in greater amounts than ordinary powdered sulfonamides from hydrophilic bases.

2. The addition of the wetting agent, sodium lauryl sulfate, to white ointment base containing 5 per cent microcrystalline sulfadiazine caused the drug to diffuse slightly from the base.

3. Microcrystalline sulfadiazine gave greater diffusion than ordinary sulfadiazine from hydrophilic ointment-type base at both room temperature and 37.5°.

4. Sulfadiazine in white ointment bases containing sodium lauryl sulfate stored for one month at 37.5° showed no diffusion.

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A Comparison of the Absorption of Radioactive Sodium Iodide from Lard, Wool Fat and Petrolatum*

By GILMAN N. CYR†, DONALD M. SKAUEN‡, JOHN E. CHRISTIAN, and C. O. LEE

The results of a study of the comparative absorption of radioactive sodium iodide from lard, wool fat, and petrolatum are reported. The results indicated that absorption of this salt was greater from white petrolatum than from either wool fat or lard. The white rat was used as the test animal.

As far back as the nineteenth century, there have appeared in the medical and pharmaceutical literature numerous articles reporting investigations on the determination of the absorption of substances through the skin and into the body. These experiments were conducted sometimes on animals and sometimes on humans, and the results leave little doubt that some substances can be made to pass through the skin in amounts that can be measured or that can produce systemic effects. However, there still exists considerable controversy concerning the absorption of some medicaments from ointment bases.

The literature reveals many methods of testing the penetration of medicinals through the skin. Lehmann (1) applied ointments containing potassium iodide to the shaven skin of puppies and was able to detect potassium iodide in their urine. In a similar manner ointments containing potassium iodide were applied to horses, dogs, sheep, and rabbits, and absorption was also demonstrated (2). Other workers (3) showed that there was a rise in blood iodine after humans were bathed in potassium iodide solutions.

Alkaloids have been used to prove that absorption occurs from certain bases. An ointment containing 2.0 Gm. of strychnine hydrochloride in 8 Gm. of lard applied to the shaven skin of a dog resulted in its death within ten minutes (4).

Vitamins also have been used as test materials for measuring absorption on both humans and lower animals. Kasahara and Kawashima (5), rubbed 1 Gm. of an ointment containing 0.3 Gm. of ascorbic acid into the breasts of four nursing mothers for twenty minutes. After one hour the ascorbic acid content of the milk showed an average gain of 1.6 mg. %, and after two to three hours returned to normal. Bruger and Flexner (6), showed that insulin was absorbed from freshly shaven skin, but not from skin that was allowed to heal for five to seven days after shaving.

An interesting approach to the problem of absorption of ointment bases themselves was presented by Czetsch-Lindenwald in 1943 (7). After the application of hydrogenated lard containing 33.6 mg. % of heavy hydrogen, deuterium oxide could not be demonstrated in the products of combustion of the body fat. Thus absorption could not be proved, and he suggested that the process be called penetration rather than absorption.

In cases where absorption of some substances could not be proved, it is possible that if more delicate methods of detection had been available, analysis of the blood or urine for these substances might have revealed some degree of absorption. Further, some substances, the absorption of which has been tested, are normally present in the body. Where their absorption was reported as a result of an increase in the content of the substance in the blood or urine, such an increase may not have been statistically significant.

A New Technique to Test Absorption.—Many radioactive isotopes can be used effectively for labeling and tracing the stable, nonradioactive isotopes. Biological processes are not able to differentiate between the common, stable isotopes and those that are radioactive. Radioactive isotopes can be detected at very much lower concentrations than the common, stable ones. Using a labeled compound, minute traces of it which have been absorbed can be detected even though the stable compound is normally present in the body.

Using sodium iodide labeled with iodine 131, a few bases were tested for their efficiency in permitting the absorption of the iodide ion through the skin of rats. This represents a new technique for testing skin absorption of various substances.

EXPERIMENTAL

Standardization of Labeled Sodium Iodide.—The sodium iodide was obtained from Oak Ridge as a solution of NaI^{131} in sodium bisulfite solution, *p*H 8.0. One-tenth of 1 cc. of this solution was diluted to 100 cc., and 1 cc. of this dilution made up to 100 cc. with 10% NaOH solution. Three portions of 0.1 cc. each of this final dilution were dried on three

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separate strips of filter paper cut to 1/2- by 2 1/2-inch dimensions and attached to a small gummed label for handling and identifying the strip. When dry, each strip was backed with Scotch tape and covered with a strip of cellophane to seal the filter strip. Each strip was placed underneath and parallel to a cylindrical Geiger-Müller counter tube (Eck and Krebs type). An aluminum plate in the counter tube holder held the strip close to and at a definite distance from the counter tube. Each strip was counted for ten minutes, the background was then subtracted, and the counts divided by 10 to give the counts per minute (CPM). The CPM for each strip were averaged. By multiplying the average activity by the proper dilution factor, the CPM per cc. of stock solution of NaI^{131} were determined. A correction for decay gave the approximate activity in CPM for the solution at any time desired.

Preparation of the Ointments.—The ointments used in these experiments were composed of

NaI	10 mg.
NaI^{131}	Approx. 10,000,000 CPM[1]
Base	90 mg.

This ointment was applied to each animal used in the tests. Since ten animals were usually tested with each base, enough ointment plus a convenient excess was made with each base. If the base did not contain water, a small portion (50 to 100 mg.) of the base was spread evenly over the concave surface of a 2-inch watch glass. To this was added the required quantity of dry, crystalline sodium iodide and the stock solution of labeled sodium iodide. The contents of the watch glass were evaporated to dryness in an oven at 100°. When dry, the material in the watch glass was transferred with a small spatula to a small glass plate. Considering the quantity (if any) of base already used to grease the watch glass, enough base was weighed on a small piece of parchment weighing paper to make the proper amount of ointment. The watch glass was "rinsed" with a portion of the base by introducing some of the base onto the glass and spreading it with the spatula. These "rinsings" were also transferred to the glass plate. The "rinsing" process was repeated twice to insure as complete a removal of the dry material as possible. When bases containing water were used, the isotope solution and the sodium iodide were evaporated to dryness in the watch glass without preliminary greasing. When dry, the material was transferred to the glass plate and the watch glass "rinsed" as described above.

The evaporated material was levigated thoroughly on the plate with some of the base. The remainder of the base was added by geometric progression, and mixed thoroughly after each addition.

Standardization of the Ointment.—Approximately 10 mg. of the ointment were accurately weighed on a small piece of parchment weighing paper and introduced on the paper into a 100-cc. volumetric flask. This portion of the ointment was dissolved in a suitable solvent. For example, for the ointment made from lard, about 50 cc. of chloroform was added to the flask to dissolve the lard. The solution was brought up to volume with alcohol which served to dissolve the sodium iodide. The result was a clear solution of all components.

[1] The weight of this material is negligible.

One-tenth of 1 cc. of 10% NaOH solution was deposited on each of three filter strips and allowed to dry. When dry, 0.1 cc. of the ointment solution just prepared was dried on each of these strips. When the strips were dry, they were sealed as explained above. The strips were counted, and the counts averaged and converted to the equivalent of 10 mg. of ointment. As seen from the dilution pattern, when this count in CPM was multiplied by 10,000, the figure obtained was the CPM applied to each animal in 100 mg. of ointment.

Preparation of the Animals.—White rats of both sexes and weighing between 130 to 300 Gm. were anesthetized with 4 mg. of pentobarbital sodium per 100 Gm. of body weight. The solution used contained 10 mg. of the drug per cc. of distilled water and was injected intraperitoneally. When the animal was anesthetized, it was placed with its chest over a cylinder 1 1/2 inches in diameter. This was to round out the animal's back and facilitate the application of the ointment and cover.

The area selected for application was situated on the animal's back about 1 inch away from the neck and a little behind the shoulders. The hair was clipped in the shape of an arc directly in back of the area. The tails of the arc pointed anteriorly. This clipped, curved line was filled with DuPont DUCO cement. A metal disk of 7/8 inch diameter was placed inside the arc and about 1/8 inch away from the arc. A ring of cement was made about 1/8 inch around the metal disk. When the cement had dried and the disk was removed, the result was a well-defined area 1 inch in diameter.

Application of the Ointment.—Portions of exactly 100 mg. of the ointment were weighed on squares of parchment weighing paper measuring 2 inches on each side. Each paper had a circle of 1 inch diameter delineated in its center in which the ointment was placed. The paper was cut around the delineated area and placed ointment-face-down in the area prepared on the animal. The paper was pressed down with a blunt object to spread the ointment over the area without rubbing.

Covering the Area.—Another ring of cement was placed about 1/4 inch away from the ring already placed on the animal's back. Another ring of cement of approximately equal diameter was applied to a piece of thin rubber sheeting measuring at least 2 inches on each side. This rubber sheeting was made of pure latex and was of the kind generally used by dentists for making rubber dams. This rubber sheet was placed cemented-face-down over the ointment area and pressed on the cemented portions with the fingers to seal it well to the animal's back. When the cement was dry and the rubber was apparently sealed to the animal's back, the excess rubber at the corners and sides was cut away.

The Animal Storage Cage.—The cage was improvised from a 4-liter beaker as follows. A circular piece of wire screen of the inner diameter of the beaker, was suspended from the rim of the beaker such that it hung about 2 or 3 inches from the bottom of the beaker. Urine and droppings fell through the screen and were collected on wood shavings on the bottom of the beaker. Food was available from a cup having a small opening at the top and resting on the wire screen. Water was available from a bottle suspended from above. A wire screen was placed over the top of the beaker and weighted down.

After each experiment, the shavings were discarded and all parts of the cage were scrubbed thoroughly to remove all contamination.

Sacrificing the Animal and Removing Samples.—The animal was sacrificed and tied down on its back to a board. The neck region was opened ventrally and the trachea was exposed. To ensure entire removal of the thyroid, a section of the trachea from the larynx down to the fourth tracheal ring was cut. This section, to which the thyroid was attached, was placed in a vial containing 1 cc. of 10% NaOH solution. The vial was stoppered tightly and allowed to stand at room temperature for twenty-four hours to dissolve the thyroid material. One-tenth of 1 cc. of this solution was evaporated and dried on a filter strip and the filter strip treated as described above.

Control Samples.—One animal was treated exactly as the test animals in each experiment, except that no ointment was applied. Counting the thyroid samples of these control animals served as a check on the presence or absence of contamination during the process. In every instance, the activity of the thyroid samples of the control animals was not significantly higher than the background.

Treatment of Results.—In order to eliminate corrections for decay, for the resolution and efficiency of the counter tube and circuit, and for other possible counting errors, the ointment standard filter strips and the thyroid sample filter strips were counted at the same time. The activity found in the entire thyroid gland was expressed in terms of percentage of the total amount of iodide applied to the skin. These calculations were made by means of the following formula:

$$\frac{T \times 10}{S \times 10{,}000} \times 100 = \% \text{ of amount of applied iodide found in entire thyroid gland,}$$

in which

T = CPM of thyroid sample
S = CPM of corrected ointment standard sample
The factors 10 and 10,000 are dilution factors

Determination of Maximum Absorption Time.—This experiment was designed to determine how long the ointments should remain on the skin to permit maximum absorption of the iodide ion. Forty rats were treated with radioactive sodium iodide in lard in the usual manner and placed in cages. At the end of ten hours, ten rats were sacrificed, the thyroids processed, counts taken, and the results tabulated. At the end of twenty, thirty, and forty hours, the process was repeated, results averaged and compared as indicated in Table I. This comparison showed that the maximum amount of absorption had taken place after about thirty hours. All ointments were, therefore, allowed to remain in contact with the animal's skin for thirty hours and the animals sacrificed.

Application of Technique and Results Obtained.—Using the method described above, ointments of labeled sodium iodide were made in lard, wool fat, and white petrolatum. Groups of about ten rats each were treated with each ointment. In all cases, the rats were sacrificed thirty hours after application of the ointment. The thyroids were removed and treated as explained above. Using the formula given, the per cent of applied iodide present in the entire thyroid was determined. For each group of rats treated, the average percentage of the amount of applied iodide found in the thyroid gland was calculated. The standard error of this mean was also calculated. The results obtained (Table II), show that while the three bases may be different in their efficiency for permitting the absorption of the iodide ion, the differences by this method do not appear to be very great.

TABLE I.—AVERAGES OF THE PER CENT OF THE AMOUNT OF APPLIED IODIDE FOUND IN THE THYROID GLAND FOR GROUPS OF RATS TREATED FOR VARYING PERIODS OF TIME WITH A 10% OINTMENT OF LABELED SODIUM IODIDE IN LARD

Group Number	Number of Animals in Group	Duration of Application (Hours)	Amount of Applied Iodide Found in Thyroid of Rats in Group, Mean %	Estimated Standard Error of the Mean
1	10	10	0.0160	0.0025
2	9	20	0.0303	0.0044
3	9	30	0.0417	0.0069
4	10	40	0.0348	0.0050

TABLE II.—AVERAGES OF THE PER CENT OF THE AMOUNT OF APPLIED IODIDE FOUND IN THE THYROID GLAND FOR GROUPS OF RATS TREATED FOR THIRTY HOURS WITH 10% OINTMENTS OF LABELED SODIUM IODIDE IN LARD, WOOL FAT, AND WHITE PETROLATUM

Base	Number of Rats Treated	Amount of Applied Iodide Found in Thyroid in Rats in Group, Mean %	Estimated Standard Error of the Mean
White petrolatum	10	0.0451	0.0032
Wool fat	10	0.0406	0.0034
Lard	9	0.0369	0.0031

SUMMARY AND CONCLUSIONS

1. The advantages of using radioactive isotopes as tracers in testing the absorption of substances through the skin has been demonstrated.

2. A technique has been developed for measuring the absorption of the iodide ion from ointment bases by tracer methods. The method developed applies to absorption through the skin of albino rats but the conclusions do not necessarily indicate that similar results would occur with the human skin.

3. The amount of radioactive sodium iodide which passes through the skin and which was absorbed by the thyroid gland was small. The highest penetration amounted to approximately 0.05 per cent of the amount applied.

4. Maximum absorption of the radioactive sodium iodide from lard took place in about thirty hours under the experimental conditions of this study. Its absorption from white petrolatum was greater than from either wool fat or lard.

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A Study of the Absorption of Radioactive Sodium Iodide from a Variety of Ointment Bases*

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A summary is presented of the results of a study of the absorption of radioactive sodium iodide from 37 ointment bases of varying composition. Absorption from half of the bases was equally as good as from lard. The other half of the bases proved to be less efficient than lard from the point of view of absorption. The salt was most efficiently absorbed from wool fat. The white rat was used as the test animal.

IN A PRECEDING paper (1), a method was presented for determining the absorption of the iodide ion through the skin of rats. The relative efficiency of lard, wool fat, and petrolatum in permitting the absorption of the iodide ion was determined.

This study was continued with two purposes in mind:

1. To determine to what extent the technique was applicable to other types of bases.

2. To determine the relative efficiency of other bases in permitting the absorption of the iodide ion.

EXPERIMENTAL

Ointments of labeled sodium iodide in lard were applied to seven groups of 9 or 10 rats each. Each group was treated at a different time. For all the groups treated, the animals were sacrificed thirty hours after application of the ointment and the thyroids removed and treated as previously explained (1). The results in Table I show that the mean and its standard error varied from group to group. A statistical test of homogeneity of variance and an analysis of variance showed that the "date" had no effect on the mean or on its variability. Thus these variations are probably inherent in the process of absorption. The accuracy of the method of measurement was checked by measuring and counting duplicate samples. Variations obtained here were too small to have any effect on the results

In order that the standard would be more valuable, the figures obtained from all the rats treated for thirty hours with the lard ointments were pooled. The mean % of applied iodide found in the thyroid glands of all the animals was calculated and used as a standard with which to compare the other bases.

Using the technique previously described (1), groups of about 10 rats each were treated with the following ointment:

NaI	10 mg.
NaI^{131}	Approx. 10,000,000 CPM.[1]
Base	90 mg.

A different base was applied to each group of animals. The mean % of the amount of applied iodide found in the thyroid glands of the animals in each group was calculated. These figures are shown in Table II. The bases are entered in the table in the order of decreasing efficiency.

DISCUSSION

In Table II it may be seen that for some bases the standard error of the mean is rather large in relation to the mean. This indicates greater variability in the mean and perhaps a less reliable mean. The reason for this can be attributed, perhaps, to the fact that the hair on the animals was not removed prior to application of the ointments. Some bases may not have been able to penetrate through the hair and to the skin in order for the labeled iodide to be

TABLE I.—AVERAGES OF THE PER CENT OF THE AMOUNT OF IODIDE FOUND IN THE THYROID GLAND FOR GROUPS OF RATS TREATED FOR 30 HOURS WITH A 10% OINTMENT OF LABELED SODIUM IODIDE IN LARD

Group Number	No. of Animals in Group	Amount of Iodide Found in Thyroid of Rats in Group, Mean %	Estimated Standard Error of Mean
1	9	0.0423	0.0050
2	10	0.0448	0.0063
3	10	0.0363	0.0045
4	9	0.0369	0.0031
5	9	0.0407	0.0058
6	10	0.0414	0.0038
7	10	0.0296	0.0034

[1] The weight of this material is negligible.

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TABLE II. AVERAGES OF THE AMOUNT OF IODIDE FOUND IN THE THYROID GLAND FOR GROUPS OF RATS TREATED FOR THIRTY HOURS WITH 10% OINTMENTS OF LABELED SODIUM IODIDE IN VARIOUS BASES

	Base	No. of Animals Treated	Applied Iodide Found in Thyroid of Rats for the Group, Mean %	Estimated Standard Error of Mean	Significance Ratio[a]
1.	Hydrous Wool Fat, U. S. P.	10	0.0499	0.0047	2.34
2.	White Petrolatum, U. S. P.	10	0.0451	0.0032	1.93
3.	Aquaphor with 50% water	10	0.0415	0.0045	0.68
4.	Anhydrous Wool Fat, U. S. P.	10	0.0406	0.0034	0.63
5.	Petrolatum, U. S. P.	9	0.0402	0.0038	0.48
6.	White Ointment, U. S. P.	9	0.0396	0.0053	0.25
7.	Hydrophylic Ointment, U. S. P.	10	0.0396	0.0030	0.40
8.	Sorg-Jones Base, 25% water (2)	9	0.0392	0.0070	0.14
9.	Sulfated Hydrogenated Castor Oil (Lard Standard)	9 66	0.0384 0.0382	0.0032 0.0017	0.06 ...
10.	Aquaphor with 25% water	9	0.0366	0.0051	0.29
11.	Purdue Hydrophylic Ointment[b]	10	0.0348	0.0047	0.67
12.	Aquaphor	9	0.0345	0.0044	0.79
13.	Aquaphor with 10% water	10	0.0339	0.0039	1.02
14.	Antiperspirant Cream[c]	8	0.0330	0.0039	1.22
15.	Cleansing Cream (3)	10	0.0327	0.0028	1.70
16.	Yellow Ointment, U. S. P.	10	0.0326	0.0024	1.92
17.	Rose Water Ointment, U. S. P.	9	0.0322	0.0034	1.57
18.	Sorg-Jones Base (2)	9	0.0320	0.0042	1.36
19.	Carbowax 1500	10	0.0311	0.0033	1.93
20.	Hydrophylic Petrolatum	10	0.0295	0.0039	2.04
21.	Soluble Ointment Base, R. B. III	8	0.0294	0.0036	2.23
22.	Ointment of Wool Alcohols, B. P.	9	0.0293	0.0028	2.75
23.	White Petrolatum with 10% talc.	10	0.0278	0.0027	3.22
24.	Hydrous Ointment B. P.	8	0.0269	0.0030	3.26
25.	Hydrogenated Oil[d]	10	0.0256	0.0033	3.40
26.	C. H. Base[e]	9	0.0231	0.0026	4.85
27.	Solid Petroxolin, N. F.	9	0.0199	0.0059	3.00
28.	Sodium Alginate 5%	8	0.0179	0.0061	3.22
29.	White Petrolatum with 10% Starch	9	0.0161	0.0055	3.87
30.	Pectin Paste, N. F.	9	0.0137	0.0064	3.70
31.	Protective Cream (4)	10	0.0122	0.0043	5.59
32.	Glycerite of Starch, U. S. P.	9	0.0112	0.0038	6.52
33.	Soluble Ointment Base, R. B. III with 10% Bentonite added	9	0.0088	0.0044	6.23
34.	Wax Ointment, R. B. III	9	0.0079	0.0026	9.81
35.	Bentonite Gelatin 20%	8	0.0012	0.00008	21.77
36.	Zinc Oxide Paste, N. F.	9	0.0004	0.00014	22.23
37.	Methyl Cellulose 1500, *CP*, 10%	7	0.00006	0.00005	22.41

[a] The figures in this column are obtained by dividing the difference between the mean for the base and lard by the standard error of that difference. A significance ratio of greater than 1.96 was chosen to indicate a significant difference in the means.
[b] Base consists of stearyl alcohol 14 Gm., white petrolatum 20 Gm., glycerin 15 cc., Duponal C 1 Gm., and distilled water 50 cc.
[c] Veto, Colgate-Palmolive-Peet Co.
[d] Dexo, The Great Atlantic and Pacific Tea Co.
[e] This base consists of white petrolatum 9 Gm., paraffin 4 Gm., liquid petrolatum 12 cc., glyceryl monostearate 16 Gm., glycerin 4 cc., and distilled water 55 cc.

available to the skin. The technique is probably lacking in that it has been impossible to obtain an *intact* hairless skin area up to now. The method, therefore, cannot be used in testing all types of bases.

SUMMARY AND CONCLUSIONS

1. The averages of the per cent of applied iodide found in the thyroid gland for groups of rats treated for thirty hours with ointments of 10 per cent labeled iodide in various bases are given.

2. With respect to the relative efficiency of these bases in permitting the absorption of the iodide ion through the skin of rats and on the basis of a significance ratio of 1.96.

(*a*) Hydrous wool fat appears to be more efficient than lard; (*b*) Bases 2 through 19 in Table II appear to be as equally efficient as lard; (*c*) Bases 20 through 38 in Table II appear to be less efficient than lard.

3. A difference in the type of emulsion, *o/w* or *w/o*, does not seem to have an appreciable effect upon the absorption of the iodide ion from emulsion bases. Such bases seem to permit absorption to a varying degree even though they may be of the same type.

4. To determine more accurately the relative efficiency of some bases the method employed here probably would require some modification.

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Note

A Note on Brucealin, the Active Principle of Ya-tan-tzu, a Chinese Antiamebic Drug*

By CHEN-YU SUNG†

YA-TAN-TZU, or the seed of *Brucea javanic* Merr., has been reported to be effective for the treatment of amebic dysentery (1–3). Many attempts have been made to isolate the active principle. Liu, Chang, and Chuan (4) reported the isolation of a white base, yatanine, as the active principle of *ya-tan-tzu*. We have applied their method of isolation but failed to obtain the compound they described. Lan (5) reported the isolation of several crystalline substances from *ya-tan-tzu*. Uno Haiki (6) obtained a bitter crystalline substance melting at 210° and a yellow powder having glycosidal properties from *ya-tan-tzu* without describing their amebacidal actions.

The purpose of this paper is to report the isolation of brucealin (so named in this laboratory), a white amorphous powder having glycosidal properties. Our method of isolation and purification is different from that of Uno Haiki and apparently our product is also purer. Its physical and chemical characteristics and its amebacidal activity *in vitro* are described.

EXPERIMENTAL

Material.—Our material was obtained from the T'ientsin market. It had been kept for several years in the drugstore. The shells were removed from the seed and only the kernels were used.

Removal of Oil.—The kernels contained more than 53% of oil which was partly removed by pressing and partly by extraction with petroleum ether. Carbon bisulfide may also be used.

Isolation and Purification of Glycoside.—Forty grams of the oil-free powder of *ya-tan-tzu* were extracted with 96% of alcohol in a Soxhlet extractor. A thick viscous fluid was obtained after the removal of alcohol by evaporation. The fluid was treated with 100 cc. of boiling water and filtered. The filtrate was washed with chloroform several times until the chloroform was no longer colored. The aqueous solution was treated with charcoal until it was only slightly colored. A dilute solution of lead acetate was added slowly until no more precipitate was formed. The solution was filtered, the excess lead was removed by H_2S. The faint yellow solution was evaporated under reduced pressure to nearly dryness. The yellow residue was kept in a desiccator over calcium chloride until it was completely dried. The crude product weighed 9 Gm.

Five grams of the powdered substance was dissolved in 10 cc. of methyl alcohol, warmed with charcoal, and filtered. A white amorphous powder was obtained by diluting the solution with two volumes of acetone. This process was repeated several times until a completely white powder was obtained. The product weighed 0.5 Gm.

Physical and Chemical Characteristics.—Brucealin is a white, amorphous hygroscopic powder with a molecular weight of about 332 (Beckmann's freezing-point method). It swells at 101° and decomposes at 140°. It is soluble in water, methyl alcohol, and hot ethyl alcohol, less soluble in cold alcohol and sparingly soluble in acetone, benzene, ether, chloroform, and most other organic solvents. Its aqueous solution is neutral to litmus and very bitter to the taste. It decolorizes dilute potassium permanganate solution and bromine water but not bromine in carbon tetrachloride. Brucealin does not reduce Fehling's solution, but after being boiled for a few minutes with a drop of hydrochloric acid solution it reduces readily. Its aqueous solution is optically active. The specific rotation, $[\alpha]_D^{25}$, is about −39.7°.

Amebacidal Activity.—A study of the amebacidal activity of Brucealin before purification through methyl alcohol and acetone was made by Dr. T. K. Chuan of the Department of Pharmacology of this college. Brucealin was active *in vitro* against *E. histolytica* in a dilution of 1:30000. The purified glycoside was tested for amebacidal activity *in vitro* against *E. histolytica* in Hansen's egg infusion medium by Dr. E. W. Dennis of the Sterling-Winthrop Research Institute. The order of activity was found to be somewhat less than that of emetine, but there was a distinct reduction in number of amoebae when the compound was tested at the dilution 1:50000.

SUMMARY

An amorphous glycosidal substance has been isolated from *ya-tan-tzu*. Its chemical and physical characteristics are described. It is active as an amebacide *in vitro*.

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Book Reviews

Laboratory Manual for Principles and Processes of Pharmacy. By HENRY M. BURLAGE, JOSEPH B. BURT, and L. WAIT RISING. McGraw-Hill Book Co., New York. 2nd ed., 1949. xvii + 271 pp. 19 x 28 cm. Price $3.

This is the usual laboratory manual designed to be used in pharmacy courses in "principles and processes." The first half of the book is a set of directions and the remainder consists of tear sheets to be filled in by the student, concerning the operation in question.

In general, the reviewer believes that this manual represents the approach desired by those who use the textbook this manual is designed to accompany. The classical content of such courses will be found here. There are certain weaknesses, however. For example, the pharmaceutical literature of recent years is filled with evidence that prescription tolerances found in many pharmacies are far too broad. Many people believe that a portion of the blame for this state of affairs resides in the colleges. Unless the exercises on weighing and measuring in this book are greatly supplemented by the instructor, the critics may have justification for their beliefs.

Many will question the section on osmotically balanced solutions for oversimplification. Although the authors are not as exact in their methods as a physical chemist would prefer them to be, the reviewer believes that, relative to the biological significance of these solutions, such simplification is desirable. It is unfortunate, however, that the authors use $-0.80°$ as the factor for the freezing point of tears, since there is such mounting evidence that, osmotically, tears are substantially equivalent to plasma.

Dentistry in Public Health. By WALTER J. PELTON and JACOB M. WISAN. W. B. Saunders Co., Philadelphia, 1949. xi + 363 pp. 15 x 23 cm. Price $5.50.

Although public health dentistry has not grown very rapidly during the past decades, today it must be recognized that it has arrived. Since dental disease is one of the most widespread of all diseases, it is fitting that something should be done on a community basis.

Since the field of dental health cuts across so many health fields, each chapter of this book was written by someone well qualified to develop its subject matter.

The first few chapters of the book deal with background and delineation of the problem. This is followed by chapters on the tools for attacking the problem and, lastly, application of the tools.

The book was written with the full cooperation of the American Public Health Association, and the Executive Secretary of this organization wrote the foreword. The book is intended as a text for students of dentistry.

Development Schedule of New Drug Products. By PAUL DE HAEN. Romaine Pierson Publishers, Inc., New York, 1949. iv + 121 pp. + extra charts. 18 x 25.5 cm. Price $15.

Product development is a highly complex form of research today, and forms the backbone of any industry—especially the drug industry.

Product development is not merely a function of the research department as the more academic mind is inclined to believe, but it is a process in which research, control, production, sales, advertising, and the medical departments all must play a role. Because so many have a stake in product development, the whole procedure must be well integrated and each participant must be aware of the need for good timing.

This book has been written to aid in bringing about such integration and timing. This is accomplished by the ingenious use of flow charts and work sheets to enable each department to keep track of its position and to guide the director of product development and/or the product development committee in keeping track of the over-all progress. Additional work sheets may be purchased from the publisher at $7.50 for a lot of 5 complete sets.

Since there is considerable variation in plan of organization from one company to another, the plans outlined here may have to be modified for specific conditions. Naturally the mere following of such a scheme cannot guarantee a "miracle" product, but certainly such a systematic scheme tends to promote efficiency.

Many people in the drug field will find this to be an interesting and stimulating book.

Drug Plants of Africa. By THOMAS S. GITHENS. University of Pennsylvania Press, Philadelphia, 1948. vii + 125 pp. 13 x 22 cm. Paper bound. Price $2.25.

This booklet is a collation of information and facts, which the author discloses are misinformative, concerning African plants used in empirical medicine. The author has evidently sifted a great deal of data in the preparation of this handbook and he is to be congratulated on the care he has given the compilation.

Much of the data presented in this booklet are in convenient tables.

Table I is a table of exports from Africa. Unfortunately too much of the data is very old, some of it covering the 19th century. Tables II and III deal with imports from Africa into the United States and England. An unnumbered table of synonyms follows. A series of tables covering several pages gives the African drugs in terms of alleged medical properties and other tables give the plant name, geographic region, plant part, "active" principle, and use. Nearly three pages of selected bibliography close the book. This is No. 8 in a series of African handbooks.

A Textbook of Colloidal Chemistry. By HARRY B. WEISER. John Wiley and Sons, Inc., New York, 2nd ed., 1949. x + 444 pp. 15 x 23 cm. Price $5.50.

Anyone wishing to learn the basic principles of colloidal behavior and to gain some insight into the practical application of colloid chemistry in the industrial arts, agriculture, and biology will find this book useful.

Many of the classical experiments of the founding fathers of colloid chemistry—Graham, Wo. Ostwald, Freundlich, Einstein, Svedberg, Hardy, Donnan, Perrin, Langmuir, Gortner, and Bancroft—are presented in an interesting fashion. To show the student that modern scholars are still contributing to this useful science, the work of such contemporaries as Florey, McBain, Debye, Mark, Hauser, and others has been incorporated.

Those familiar with Freundlich's *Kapillarchemie* will find in this work the book Freundlich himself would probably have written if he were writing a modern textbook. The examples are chosen with care, the explanations are clear, and the right amount of mathematics has been used. The references at the end of each chapter were evidently selected with care.

The applied area has been treated somewhat cursorily, but there are many books available on application.

1950 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1950 Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1950 Award, nominations must be received on or before January 1, 1950.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During a period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Iodine Educational Bureau, Inc.

The award consists of $1000 and a diploma setting forth the reasons for selection of the recipient. It may be presented annually at the annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council and which functions under prescribed rules. The present committee includes Justin L. Powers, chairman; Louis Gershenfeld, Charles O. Wilson, Harvey B. Haag, Heber W. Youngken, John C. Krantz, and Frank O. Taylor.

The award is now in its third year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist. Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, will be the recipient of the second award and will give the 1950 Iodine Award lecture.

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